<table>
<thead>
<tr>
<th>Paper ID</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>P345</td>
<td>Immune recovery in acute and chronic HIV infection and the impact of thymic stromal lymphopoietin</td>
<td>Gelpi, M*; Hartling, H; Thorsteinsson, K; Gerstoft, J; Ullum, H; Nielsen, S (Copenhagen, Denmark)</td>
</tr>
<tr>
<td>P347</td>
<td>Cardiovascular risk in HIV-positive subjects: analyses of T-cell phenotype and CD49d expression</td>
<td>Zingaropoli, M*; D’Abramo, A; Iannetta, M; Oliva, A; d’Ettorre, G; Lichtner, M; Mastroianni, C; Ciardi, M; Vullo, V (Rome, Italy)</td>
</tr>
<tr>
<td>P348</td>
<td>Baseline myeloid and lymphoid activation markers can predict time to viral load reduction under 50 copies/mL and CD4 recovery, respectively, after highly-active antiretroviral therapy initiation</td>
<td>Iannetta, M*; Lichtner, M; Rossi, R; Savinelli, S; Vita, S; Mascia, C; Zuccalà, P; Marocco, R; Zingaropoli, M; Ciardi, M; d’Ettorre, G; Mastroianni, C; Vullo, V (Rome, Italy)</td>
</tr>
<tr>
<td>P349</td>
<td>Impact of oestrogen plasma levels in modulation of immune activation among HIV-infected women and men undergoing successful antiretroviral therapy</td>
<td>Marrócco, R*; Lichtner, M; Tieghi, T; Belvisi, V; Pozzetto, I; Mascia, C; Zuccalà, P; Rossi, R; Mengoni, F; Mastroianni, C; Villo, V (Rome, Italy)</td>
</tr>
<tr>
<td>P351</td>
<td>Geno2pheno [coreceptor-hiv2]: a new diagnostic tool for the genotypic determination of HIV-2 coreceptor usage</td>
<td>Döring, M*; Borrego, P; Büch, J; Martins, A; Friedrich, G; Camacho, R; Eberle, J; Kaiser, R; Lengauer, T; Taveira, N; Fiefe, N (Saarbrücken, Germany)</td>
</tr>
<tr>
<td>P352</td>
<td>High rates of multi-class drug resistance in HIV-1-infected individuals monitored with CD4 cell count in Uganda</td>
<td>von Braun, A*; Scherrer, A; Sekaggya, C; Kirangwa, J; Ssemwanga, D; Kaleebu, P; Günthard, H; Kambugu, A; Castelnuovo, B; Fehr, J (Kampala, Uganda)</td>
</tr>
<tr>
<td>P353</td>
<td>Prevalence and impact of transmitted drug resistance in recent HIV-1 infections, Germany 2013–2015</td>
<td>Hauser, A*; Hofmann, A; Hanke, K; Bremer, V; Bartmeyer, B; Kücherer, C; Bannert, N (Berlin, Germany)</td>
</tr>
<tr>
<td>P354</td>
<td>Higher rates for transmission of NNRTI-resistant viruses for subtype A versus subtype B strains in Southern Greece</td>
<td>Kostaki, E; Sypsa, V; Nikolopoulos, G; Gargalianos, P; Xylomenos, G; Lazanas, M; Chini, M; Skoutelis, A; Papastamopoulos, V; Antoniadou, A; Papadopoulos, A; Psychogiou, M; Daikos, G; Chrysos, G; Paparizos, V; Koukouriti, S; Sambatakou, H; Sipsas, N; Lada, M; Panagopoulos, P; Matteos, E; Hatzakis, A; Paraskevis, D* (Athens, Greece)</td>
</tr>
<tr>
<td>P356</td>
<td>Low prevalence of pre-treatment HIV-1 drug resistance in Ugandan adults</td>
<td>von Braun, A*; Sekaggya, C; Scherrer, A; Magambo, B; Ssemwanga, D; Kaleebu, P; Günthard, H; Kambugu, A; Fehr, J; Castelnuovo, B (Kampala, Uganda)</td>
</tr>
<tr>
<td>P357</td>
<td>Prevalence of resistance mutations to rilpivirine and etravirine in people starting antiretrovirals in Argentina</td>
<td>Bissio, E*; Barbás, M; Bouzas, M; Cudolá, A; Falistocco, C; Salomón, H (Buenos Aires, Argentina)</td>
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<tr>
<td>P358</td>
<td>Frequency of additional resistance relevant mutations in 2% and 1% population proportions in next-generation sequencing (NGS) in routine HIV-1 resistance diagnostics</td>
<td>Ehret, R*; Moritz, A; Schuetz, M; Obermeier, M (Berlin, Germany)</td>
</tr>
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</table>
| P359 | Impact of baseline NNRTI resistance in antiretroviral-naïve patients in a large urban clinic  
|      | Steinberg, S*; Crouzet, F; Sandler, I; Varriano, B; Smith, G; Kovacs, C; Brunetta, J; Chang, B;  
|      | Merkley, B; Tilley, D; Fletcher, D; Acsai, M; Knox, D; Sharma, M; Loutfy, M (Toronto, Canada) |
| P360 | Enhanced surveillance to study HIV-1 drug resistance among naïve individuals in Southern Greece: the added value of molecular epidemiology to public health  
|      | Paraskevis, D*; Kostaki, E; Magiorkinis, E; Gargalianos, P; Xylomenos, G; Lazanas, M; Chini, M;  
|      | Skoutelis, A; Papastamopoulos, V; Antoniadou, A; Papadopoulos, A; Psychogiou, M; Daikos, G;  
|      | Zavitsanou, A; Chrysos, G; Paparizos, V; Kourkounti, S; Oikonomopoulou, M; Sambatakou, H;  
|      | Sipsas, N; Lada, M; Panagopoulos, P; Maltezos, E; Drimis, S; Hatzakis, A (Athens, Greece) |
| P361 | Transmission patterns of HIV-1 subtype A resistant strains across Greece: evidence for country and regional level transmission networks  
|      | Paraskevis, D*; Skoura, L; Kostaki, E; Gargalianos, P; Xylomenos, G; Lazanas, M; Chini, M; Metallidis, S;  
|      | Skoutelis, A; Papastamopoulos, V; Antoniadou, A; Papadopoulos, A; Psychogiou, M; Daikos, G;  
|      | Pilalas, D; Zavitsanou, A; Chrysos, G; Paparizos, V; Kourkounti, S; Chatzidimitriou, D; Sambatakou, H;  
|      | Sipsas, N; Lada, M; Panagopoulos, P; Maltezos, E; Drimis, S; Hatzakis, A (Athens, Greece) |
| P362 | Occurrence and risk factors for primary integrase resistance-associated mutations in Austria in the years 2008–2013  
|      | Zoufaly, A*; Kraft, C; Schmidbauer, C; Puchhammer, E (Vienna, Austria) |
| P363 | Transmission of HIV-1 drug resistance in Tel Aviv, Israel, 2010–2015  
|      | Turner, D*; Girshengorn, S; Braun, A; Tau, L; Leshno, A; Alon, D; Pupko, T; Zeldis, I; Matus, N;  
|      | Gielman, S; Ahsanov, S; Schweitzer, I; Avidor, B (Tel Aviv, Israel) |
| P364 | Development of T66I-mediated integrase inhibitor cross-resistance against elvitegravir under dolutegravir-containing first-line therapy  
|      | Wiesmann, F*; Däumer, M; Naeth, G; Schweitzer, H; Braun, P; Rump, J (Aachen, Germany) |
| P365 | Patterns of emergent resistance-associated mutations after initiation of non-nucleoside reverse-transcriptase inhibitor-containing regimens in Taiwan: a multicenter cohort study  
|      | Cheng, C*; Su, Y; Tsai, M; Yang, C; Liu, W; Cheng, S; Sun, H; Hung, C; Chang, S (Taoyuan, Taiwan) |
| P366 | Association of therapeutic failure with low-level viremia in HIV-infected patients in the Arevir/RESINA cohort in Germany  
|      | Lübbe, N*; Pironi, A; Knops, E; Schütler, E; Jensen, B; Oette, M; Esser, S; Lengauer, T; Kaiser, R (Düsseldorf, Germany) |
| P367 | Drug resistance mutations (DRM) among pregnant HIV-positive women in the Duesseldorf University Hospital, Germany, 2009–2016  
|      | Haars, U*; Luebke, N; Jensen, B; Haeussinger, D (Essen, Germany) |
| P368 | Prevalence of HIV type 1 drug resistance mutations in treatment-naïve patients participating in the GARDEL study  
|      | Figueroa, M*; Patterson, P; Cahn, P; Andrade-Villanueva, J; Arribas, J; Gatell, J; Lama, J; Norton, M; Sierra Madero, J; Sued, O; Rolen, M (Buenos Aires, Argentina) |
| P369 | High prevalence of transmitted antiretroviral drug resistance in newly HIV-1 diagnosed Cuban patients  
|      | Perez Santos, L*; Machado, L; Kouri Cardella, V; Diaz, H; Aragones, C; Aleman, Y; Silva, E; Correa, C; Blanco de Armas, M; Perez, J; Dubed, M; Soto, Y; Ruiz, N; Limia, C; Nibot, C; Valdés, N; Ortega, M; Romay, D; Baños, Y; Rivero, B; Campos, J (Havana, Cuba) |
P370  Viroseq protocol optimized for the detection of HIV-1 drug mutations in patients with low viral load
Monteiro, F*; Tavares, G; Ferreira, M; Amorim, A; Bastos, P; Rocha, C; Hortelão, D; Vaz, C; Koch, C; Araújo, F; Serrão, R; Sarmento, A (Porto, Portugal)

P371  The role of presepsin (sCD14-ST) as an indirect marker of microbial translocation and immune activation
Paola, C*; Zuccaro, V; Cima, S; Sacchi, P; Bruno, R (Pavia, Italy)

P372  CRF19_cpx variant emergence in a cluster in naïve patients of southern Spain: clinical and phylogenetic characterization
González-Domenech, C*; Viciana, I; Mayorga, M; Palacios, R; de la Torre, J; Jarilla, F; Castaño, M; del Arco, A; Márquez, M; Clavijo, E; Santos, J (Málaga, Spain)

P373  One-step real-time PCR for HIV-2 group A and B RNA plasma viral load in LightCycler 2.0
Bastos, P; Monteiro, F*; Tavares, G; Amorim, A; Ferreira, M; Hortelão, D; Rocha, C; Vaz, C; Koch, C; Araújo, F; Serrão, R; Sarmento, A (Porto, Portugal)

P374  The association between high pre-HAART CD8 cell counts and poorer immunological outcome following antiretroviral therapy
Wong, C*; Wong, N; Lee, S (Hong Kong, Hong Kong)
Immune recovery in acute and chronic HIV infection and the impact of thymic stromal lymphopoietin

Marco Gelpi1, Hans J. Hartling1, Kristina Thorsteinsson2, Jan Gerstoft1, Henrik Ullum3, Susanne D. Nielsen1

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Background
Symptomatic primary HIV infection is associated with faster decline in CD4+ T cells count and progression to AIDS, and immediate initiation of combination antiretroviral therapy (cART) is recommended. However, little is known about immunological predictors of immune recovery.

Thymic Stromal Lymphopoietin (TSLP) is a cytokine that promotes homeostatic polyclonal proliferation of CD4+ T cells and participates in regulating Th17/regulatory T-cell balance, immunological functions known to be affected during primary HIV infection. The aim of this study was to describe immune recovery in primary and chronic HIV infection and possible impact of TSLP.

Materials and Methods
Prospective study including 100 HIV-infected individuals (primary HIV infection (N=14), early presenters (>350 CD4+ T cells/μL, N=42), late presenters without advanced disease (200-350 CD4+ T cells/μL, N=24) and late presenters with advanced disease (>200 CD4+ T cells/μL, N=20)(Table1). Plasma TSLP was determined using ELISA and CD4+ T cell subpopulations (recent thymic emigrants, naïve, and memory cells) were measured using flow cytometry at baseline and after 6, 12, and 24 months of cART.

TSLP was associated with lower CD4+ T cell recovery in the late presenters with advanced disease whereas differences in absolute counts and proportions of CD4+ T cell subpopulations were found between primary HIV infection and late presenters supporting early initiation of cART. Higher plasma TSLP was found in primary HIV infection. Finally, higher plasma TSLP was associated with lower CD4+ T cell recovery in the late presenters non advanced disease group (correlation coefficient -0.50, P = 0.034).

Comparing the four HIV groups by using Kruskal-Wallis test. If significant (<0.05) then Mann-Whitney was used to compare PHI group with the other chronic groups. Significant differences are marked: a: PHI vs. late presenters with advanced disease; b: PHI vs. late presenters without advanced disease; c: PHI vs. early presenters.

Conclusions
Immune recovery was comparable in primary and chronic HIV infection whereas differences in absolute counts and proportions of CD4+ T cell subpopulations were found between primary HIV infection and late presenters supporting early initiation of cART. Higher plasma TSLP was found in primary HIV infection. Association between TSLP and a lower thymic output, but not with immune recovery was found in primary HIV infection. These findings indicate a possible role of TSLP in immune homeostasis in HIV infection but do not support TSLP to affect immune recovery in primary HIV infection.

Results
Immune recovery was comparable in all groups, and no differences in immune homeostasis were found between primary HIV infection and early presenters. In primary HIV infection group, lower thymic output compared to late presenters without advanced disease was found. However, lower proportion of effector memory and higher proportion of late differentiated CD4+ T cell were found in primary HIV infection compared to late presenters. TSLP was elevated in primary HIV infection at baseline and after 24 months of cART (Table2). Interestingly, TSLP was negatively associated with proportion of recent thymic emigrants (correlation coefficient -0.50, P=0.034). However, TSLP was not associated with immune recovery in primary HIV infection. Finally, higher plasma TSLP was associated with lower CD4+ T cell recovery in the late presenters non advanced disease group (correlation coefficient -0.50, P = 0.034).

Figure 1. CD4 count (A), immune recovery (B) and plasma TSLP (C) before cART and during 24 months of follow-up

Immune recovery was comparable in primary and chronic HIV infection whereas differences in absolute counts and proportions of CD4+ T cell subpopulations were found between primary HIV infection and late presenters supporting early initiation of cART. Higher plasma TSLP was found in primary HIV infection. Association between TSLP and a lower thymic output, but not with immune recovery was found in primary HIV infection. These findings indicate a possible role of TSLP in immune homeostasis in HIV infection but do not support TSLP to affect immune recovery in primary HIV infection.

Contact: marco.gelpi@regionh.dk
Cardiovascular risk in HIV positive subjects: analyses of T cell phenotype and CD49d expression

Zingaropoli, Maria Antonella; Iannetta, Marco; D’Abramo, Alessandra; Oliva, Alessandra; d’Ettorre, Gabriella; Lichtner, Miriam; Mastroianni, Claudio Maria; Ciardi, Maria Rosa; Vullo, Vincenzo

Department of Public Health and Infectious Diseases, Sapienza Rome Italy

It is well known that HIV positive subjects have a higher risk of non-AIDS-related comorbidities than general population. Chronic immune activation of T-cells plays an important role in HIV pathogenesis and related comorbidities. In this context, the integrin-α4 (CD49d), a transmembrane co-stimulatory molecule, is involved in the lymphocyte homing from peripheral compartment to the gut (α4β7) and to the central nervous system (α4β1).

Aim of the study was to evaluate CD49d expression in T-lymphocyte subsets and the relationship with cardiovascular damage in HIV positive individuals on effective combined antiretroviral therapy (c-ART).

Materials and methods

Thirty HIV positive subjects (6 females/24 males) with a mean age (± standard deviation [SD]) of 52±10.1 years on effective c-ART and 15 age and sex matched healthy donors (HD) were enrolled. T-lymphocyte immunophenotype and CD49d expression, (measured as median fluorescence intensity [MFI]), were assessed by flow cytometry (Figure 1). Carotid-Intima Media Thickness (c-IMT) was measured with ultrasonography. Normal and pathological c-IMT were defined as IMT<0.9 mm and >0.9 mm, respectively.

In animal models a potential role of CD49d in macrophages activation has been demonstrated. In this study, the increase of CD49d expression in T-lymphocytes could be considered as a marker of immuneaetivation during HIV infection. Furthermore, CD49d could represent a potential therapeutic target for the immune system modulation in the context of HIV infection aiming to reduce non-AIDS related comorbidities, especially cardiovascular diseases.
Baseline myeloid and lymphoid activation markers can predict time to viral load reduction under 50 copies/ml and CD4 recovery, respectively, after highly active antiretroviral therapy initiation

Iannetta Marco, Lichtner Miriam, Rossi Raffaella, Savinelli Stefano, Vita Serena, Mascia Claudia, Zuccalà Paola, Marocco Raffaella, Zingaropoli Maria Antonella, Ciaraldi Maria Rosa, d’Ettorre Gabriella, Mastroianni Claudio Maria, Vullo Vincenzo
Sapienza University, Department of Public Health and Infectious Diseases, Rome, Italy

P348

Background

During HIV infection myeloid and lymphoid activation has been reported, together with elevation of monocyte/macrophage inflammation markers, such as soluble (s)CD14 and sCD163. The advent of highly active antiretroviral (ARV) therapies improved both life expectancy and quality of life of persons with HIV. However, the persistence of the virus in the host leads to a state of chronic activation of the immune system, not completely reversed by ARV treatment. We evaluated both myeloid and lymphoid activation markers and correlated them with CD4 recovery after 12 months of antiretroviral (ARV) treatment and the time (in days) needed to achieve a viral load below 50 copies/ml.

Materials and Methods

Treatment-naive HIV+ patients were enrolled and followed up for one year after treatment initiation. Blood samples were collected before treatment initiation (T0). Monocyte (Mo), dendritic cell (DC) and T lymphocyte phenotypes were assessed by flow-cytometry using a lyse-no-wash protocol (gating strategy is shown in Figure 1). sCD14 and sCD163 were measured in plasma with ELISA. Seventeen age and sex matched healthy donors (HD) were included. Statistical analysis was performed with the software GraphPad Prism version 6.0.

Results

We recruited 34 naive patients (8 women, 9 AIDS presenters). 15, 10 and 9 patients started an ARV therapy containing a protease, a non-nucleoside reverse-transcriptase and an integrase strand transfer inhibitor (PI, NNRTI, INSTI), respectively. Three patients did not start any treatment (1 elite controller and 2 long-term non-progressors). No differences in HIV viral load and CD4 cell counts were observed at T0, stratifying patients according to ARV therapy.

Conclusions

The Kaplan-Meier analysis showed that higher baseline CD4+CD161+ Mo counts were predictive of a lower rate of subjects with a viral load<50 copies/ml, within 150 days from ARV therapy initiation (p=0.03) (Figure 6A). After one year of ARV therapy, CD4 recovery positively correlated with basal levels of CD8 immune-activation (Figure 6B), while the choice of treating patients with a PI, NNRTI or INSTI did not affect CD4 recovery. The three patients who did not receive any ARV treatment were excluded from the analysis.

Table 1: Clinical characteristic of the study population

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<tr>
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<th>HIV+</th>
<th>HD</th>
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<tbody>
<tr>
<td>Number</td>
<td>34</td>
<td>17</td>
</tr>
<tr>
<td>Age: median</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>[IQR]</td>
<td>[28-44]</td>
<td>[30-49]</td>
</tr>
<tr>
<td>Sex: M/F</td>
<td>26/8</td>
<td>13/4</td>
</tr>
<tr>
<td>VL: median log/ml</td>
<td>4.9log/ml</td>
<td>[4.1-5.5]</td>
</tr>
<tr>
<td>[IQR]</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td># CD4: median</td>
<td>434cells/µl</td>
<td>[101-656]</td>
</tr>
<tr>
<td>[IQR]</td>
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<td></td>
</tr>
<tr>
<td>[IQR]</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>ARV treatment</td>
<td>PI/NNRTI/INSTI</td>
<td>15/10/6</td>
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<tr>
<td>[IQR]</td>
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Table 2: Correlation between HIV-1 viral load and immunological parameters at T0

<table>
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<tr>
<th></th>
<th>pDC</th>
<th>slanDC</th>
<th>CD14+CD16++CD16+</th>
<th>CD4/HLA-DR-CD38*</th>
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</thead>
<tbody>
<tr>
<td><strong>p</strong></td>
<td>0.047</td>
<td>0.002</td>
<td>0.036</td>
<td>0.002</td>
</tr>
<tr>
<td>[IQR]</td>
<td>0.34</td>
<td>0.52</td>
<td>+0.36</td>
<td>+0.50</td>
</tr>
<tr>
<td>% of CD8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[IQR]</td>
<td>40</td>
<td>30</td>
<td>40</td>
<td>30</td>
</tr>
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</table>

CDC-C HIV+ patients showed lower levels of mDC, pDC, slanDC and CD4 immuneactivation than HD. No differences in CD8 immuneactivation levels and intermediate monocyte cell counts were observed.

HIV-1 viremia negatively correlated with pDC and slanDC cell counts and positively correlated with CD14+CD16+ Mo cell counts and CD4 immune-activation levels (table 2).

Figure 1: Gating strategy

A) Gating strategy for monocytes and dendritic cells: after doublets exclusion, lineage (Lin),CD56, CD19, CD3, CD235a) and HLA-DR+ events were gated. According to CD14 and CD16 expression monocyte were defined as classical (CD14++CD16+) intermediate (CD14+CD16+) and non-classical (CD14+CD16+). Slan-DC were identified in the CD14+CD16+ gate and defined as CD11c+ and MDC+ (CD11c+ and MDC+ (CD11c+ and MDC+). Myeloid dendritic cells (mDC) and plasmacytoid dendritic cells (pDC) were identified in the CD14+CD16+ gate and defined as CD11c-DR+ and CD11c-DR-+ respectively.

B) Gating strategy for T lymphocyte immuneactivation: after doublets exclusion, CD4+CD45+ and CD8+CD45+ lymphocytes were gated. Immuneactivated CD4 and CD8 T lymphocyte were defined as HLA-DR-CD38*.

Figure 2: pDC, slanDC and intermediate Monocytes

Comparison of pDC, slanDC and intermediate monocytes (CD14+CD16+), Mo, between treatment naive HIV+ subjects and Healthy Donors (HD).

Figure 3: T lymphocyte immuneactivation

Comparison of immuneactivation levels of CD4 and CD8 T lymphocyte, between treatment naive HIV+ subjects and Healthy Donors (HD).

Figure 4: Soluble inflammation markers

Myeloid activation soluble markers sCD14 and sCD163 were higher in HIV+ subjects compared to HD (2163 vs 1363ng/ml p<0.001 and 272,6 vs 149,1ng/ml p=0.085) (Figure 4). CD4+CD16+ Mo and CD8 immune-activation were not correlated with the clinical stage of HIV subjects, while pDC, mDC and slanDC cell counts were lower in AIDS than non-AIDS presenters. CD4 immuneactivation levels were higher in AIDS than non-AIDS presenter (Figure 5).

Figure 5: Immunological parameters in AIDS and non-AIDS presenters

The Kaplan-Meier analysis showed that higher baseline CD4+CD161+ Mo counts were predictive of a lower rate of subjects with a viral load<50 copies/ml, within 150 days from ARV therapy initiation (p=0.03) (Figure 6A). After one year of ARV therapy, CD4 recovery positively correlated with basal levels of CD8 immune-activation (Figure 6B), while the choice of treating patients with a PI, NNRTI or INSTI did not affect CD4 recovery. The three patients who did not receive any ARV treatment were excluded from the analysis.

Figure 6: Predictive value of CD4+CD161+ Mo and CD8 immuneactivation

A) Higher CD4+CD161+ Mo counts were associated to a lower rate of subjects with a viral load under 50 copies/ml, after ARV treatment initiation. The cut-off of 16.000 cell/ml represents the highest value observed in the control group.

B) CD4 recovery after 12 months of ARV treatment positively correlated with baseline CD8 immune-activation levels (Spearman r=0.50 and p<0.005).

Table 2: Correlation between HIV-1 viral load and immunological parameters at T0

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<th>slanDC</th>
<th>CD14+CD16++CD16+</th>
<th>CD4/HLA-DR-CD38*</th>
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<td>0.036</td>
<td>0.002</td>
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<tr>
<td>[IQR]</td>
<td>0.34</td>
<td>0.52</td>
<td>+0.36</td>
<td>+0.50</td>
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<tr>
<td>% of CD8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>[IQR]</td>
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P348


Contacts: marco.iannetta@uniroma1.it, miriam.lichtner@uniroma1.it
Impact of oestrogen plasma levels in modulation of immune activation among HIV-infected women and men undergoing successful antiretroviral therapy


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2 Public Health and Infectious Diseases, Sapienza University, Rome, Italy

Background:
Several sex differences have been described in the natural course of HIV-1 disease. Higher levels of TLR 7-mediated INF-alpha production together with greater levels of activated CD8-T cells were described in women compared with men for given HIV viral load. The role of sexual hormones in ART treated women is not completely understood and seem to be crucial to individualize possible eradication strategy in women that could be different that in men.
The aim of this study was to investigate the role of sexual hormones in determining innate immunity and immune activation in a cohort of HIV infected subjects undergoing effective antiretroviral treatment.

Study population

<table>
<thead>
<tr>
<th></th>
<th>WOMEN</th>
<th>MEN</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>50 y (24-76)</td>
<td>48 y(23-70)</td>
<td>0.33</td>
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<tr>
<td>CD4-Nadir</td>
<td>215 cell/mm (4-640)</td>
<td>173 cell/mm (8-472)</td>
<td>0.45</td>
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<tr>
<td>CD4+</td>
<td>660 cell/mm (178-1425)</td>
<td>709 cell/mm (243-1550)</td>
<td>0.69</td>
</tr>
<tr>
<td>HIV-RNA Zenith cp/ml</td>
<td>60779 cp/ml</td>
<td>96000 cp/ml</td>
<td>0.26</td>
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<tr>
<td>HIV-RNA</td>
<td>&lt;20 cp/ml</td>
<td>&lt;20 cp/ml</td>
<td>NS</td>
</tr>
<tr>
<td>Infection Years</td>
<td>16 y (3-27)</td>
<td>18 y (1-28)</td>
<td>0.54</td>
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<tr>
<td>Therapy</td>
<td>INSTI+ PI</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>PI</td>
<td>75</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>INNRTI</td>
<td>17</td>
<td>13</td>
</tr>
</tbody>
</table>

Methods:

✓ Whole blood samples evaluating mDC, pDC, SlanDC and typical, atypical and intermediate monocytes with a cytofluorimetric method based on 7 fluorochromes
✓ HLA-DR/CD38 CD4 and CD8 T lymphocytes were also evaluated.
✓ sCD14 and sCD163 level (pg/ml) were measured by ELISA.
✓ Sex hormones (oestradiol, progesterone, testosterone) were using CLIA kit.
✓ Non parametric Mann-Whitney test and Spearman coefficient correlation were used.

Results:

✓ No significant differences in levels of circulating dendritic cell (mDC, pDC) between HIV+ women and men.
✓ A positive correlation was found between mDC and serum oestradiol (p=0,03, r=0,30)
✓ A trend of increased number of atypical inflammatory monocytes and MDC-8 in women.
✓ A significant augmentation of DR+38+CD4+ T cells was found in men (p=0,02) and a negative correlation between DR+38+CD8+ T and serum oestradiol levels in all HIV subjects and in women was observed (respectively p=0,002; r-0,67; p=0,006, r=-0,50).
✓ Only in women a negative correlation between mDC and DR+38+CD8+ T cell was found(p=0,02; r=-0,43).
✓ Regarding soluble markers of monocytes activation, we didn’t observe differences: women have a lower levels of CD14 than men (pg/ml, median 2249 and 2685 pg/ml).

Conclusions:

In HIV aviremic ART treated subjects, high levels of oestrogen seem to be associated to an expansion of mDC and lower activation of CD8 T cells, underlying the importance of consider hormonal status and not only gender and age in designing immunological and therapeutic studies.
geno2pheno[coreceptor-hiv2]

a new diagnostic tool for the genotypic determination of HIV-2 coreceptor usage

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Relevance of HIV-2 coreceptor usage

The selection of HIV-2 variants using the CXCR4 coreceptor (X4-capable) should be prevented because X4-capable variants are harder to neutralize than viruses using only CCR5 (R5)[1].

Before prescribing CCR5-coreceptor antagonists to patients infected with HIV-2, clinicians should rule out the existence of X4-capable variants.

Goal: differentiate R5 and X4-capable HIV-2 variants based on the amino acid sequence of the V3 loop.

Materials and methods

Support vector machines (SVMs) were trained on a data set of 73 R5 and 52 X4-capable samples to classify binary-encoded V3 amino acid sequences as either R5 or X4-capable. Classifier performance was evaluated using 10-fold nested cross-validation (CV). The predicted probabilities indicating whether a sequence originates from an X4-capable variant were transformed to false positive rates (FPRs).

We developed a visual representation of position-specific classifier weights to indicate amino acids associated with R5 and X4-capable variants (see Fig. 2). We evaluated established discriminatory sequence features from a rules-based approach by Visseaux et al. [2] and novel features detected by the SVM using Fisher’s exact test with multiple testing correction (Benjamini and Hochberg).

Results

- A linear SVM (AUC=0.95) outperformed other models and was used in all subsequent analyses.
- For a set of 126 V3 sequences, the 10-fold nested CV sensitivity was 76.9% and the specificity was 97.3%.
- All samples from a set of nine, newly phenotyped V3 sequences were classified correctly by the SVM.
- We validated existing markers for X4-capability [2] and identified new, significant features (p ≤ 0.05): variants 27K, 15G, and 8S.

Visualization of model weights

Figure 2: SVM weights for the V3 loop of a ROD10 isolate.

Highlights of the tool

- Accuracy: high sensitivity and specificity
- Interpretability: visualization of sequence-specific weights and output of FPRs
- Availability: an online web service is available at coreceptor-hiv2.geno2pheno.org
- Opportunities: enables large-scale epidemiological studies on HIV-2 coreceptor usage

References

**Background**

Until a recent change in guidelines, HIV-infected patients on antiretroviral therapy (ART) in Uganda were monitored using CD4 cell counts only. So far, little is known about prevalence of drug resistance among HIV-infected patients with virological failure (VF) after immunological treatment monitoring in Uganda.

**Methods**

From June 4th – September 30th, 2015, HIV-RNA was measured in HIV-infected adults (≥18 years) on ART for at least 6 months presenting to the Infectious Diseases Institute in Kampala. In case of VF (>1000 copies/mL), HIV genotyping was requested. Sequencing of partial polymerase gene was conducted using an in-house protocol. All sequences were submitted to the Stanford University HIV Drug Resistance database and the surveillance drug resistance mutations were identified using the 2009 WHO mutations list. HIV-1 subtypes were determined using REGA version 3.0.

**Results**

HIV-RNA measurements were done in 2511 patients, who had been on ART for a median time of 4.7 years (interquartile range (IQR) 2.5-8.7). A total of 199 patients (7.9%) had VF with a median viral load of 4.4 log10 copies/mL (IQR:3.9-4.9). The majority of patients with VF (140, 70.4%) were on first-line ART, 138 (69.3%) were female, and the median age was 37 years (IQR:30-43). HIV genotyping tests were available in 163 (81.9%). HIV-1 subtypes A (46%) and D (34%) were most common. Relevant drug resistance mutations were observed in 135 (82.8%) (Figure), of which 103 (63.2%) had resistance to two drug classes, and 11 (6.8%) had resistance to all three drug classes available in Uganda.

**Conclusions**

With 92% of all patients virologically suppressed, the overall prevalence of VF was low, and is in-line with the third of the 90-90-90 UNAIDS targets. However, the majority of failing patients had developed resistance to more than one drug class, suggesting that failing regimens – not identified as such by CD4 monitoring - had been in place for a prolonged period of time. This is a call for action to get access to close virological monitoring for patients on ART, as well as access to new antiretroviral drugs such as integrase inhibitors.

**Acknowledgements:** We would like to acknowledge all patients and their families.

**Funding:** Swiss HIV Cohort Study, Gilead Sciences
Transmitted drug resistance (TDR) in new HIV-infections has significant clinical consequences for the treatment success. Therefore, monitoring of TDR in currently circulating HIV-strains is an important public health issue of the Robert Koch-Institute.

## Objective

### Predicted susceptibility to antiretroviral drugs with respect to levels of resistance in the study population.

80% (82/102) of all NRTI-selected mutations were thymidine analogue mutations (TAMs) and T215 revertants: M41L, K219NQR, D67EG, T215Y, K70R, L210W and T215CDEIS, conferring low/intermediate resistance to zidovudine (AZT) and stavudine (D4T). 60% (38/64) of NNRTI mutations M41L and V82FL are associated with low/intermediate resistance to tipranavir (TPV), nefavir (NFV) and fosamprenavir (FPV) (Figure 4+5).

### Conclusion

TDR prevalence in recent HIV-1 infections among newly diagnosed cases in Germany (2013–2015) remained high (>10%) and is comparable to other European countries. TDR was mainly caused by the first-generation NNRTI-selected K103NS, by long-term persisting TAMs and the PI-selected M46IL and V82FL. For the selection of first-line regimens (EACS V8.0), the prevalence of TDR mutations was 5.4% (0.8% NRTI; 3.1% NNRTI; 0.6% PI; 0.9% multi drug resistance; N= 12, 45, 9, 13, respectively) (Figure 6).

Considering only primary resistance mutations which impact drugs currently recommended in first-line regimens (EACS V8.0), the prevalence of TDR mutations was 5.4% (0.8% NRTI; 3.1% NNRTI; 0.6% PI; 0.9% multi drug resistance; N= 12, 45, 9, 13, respectively) (Figure 6).

\[ \text{Prevalence} = \frac{\text{Number of TDR mutations}}{\text{Total number of mutations}} \times 100 \]

\[ \text{Impact} = \frac{\text{Number of TDR mutations impacting initial regimen}}{\text{Total number of TDR mutations}} \times 100 \]
Higher rates for transmission of NNRTI resistant viruses for subtype A versus subtype B strains in Southern Greece


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Introduction

We have previously found that the most prevalent NNRTI resistant mutations among drug naïve individuals in Southern Greece were E138A and K103N

Aim

Our aim was to estimate the transmission dynamics of E138A and K103N resistant strains and to investigate for potential differences between subtypes A and B

Materials and Methods

We analyzed all sequences with E138A from 179 and 68 HIV-1 treatment naïve individuals sampled in Southern Greece during 01/01/2003 - 31/06/2015 infected with subtype A and B, respectively. Similarly we analyzed 56 and 18 sequences with K103N from subtypes A and B. Sequences were available in the PT/RT

Phylogenetic analyses were performed using a Bayesian approach as implemented in BEASTv1.8, by using the HKY as nucleotide substitution model with gamma (Γ) heterogeneity model, an uncorrelated lognormal relaxed clock model with TipDates and the birth-death basic reproductive number models (BDM). Non-informative priors were used for the MCMC runs. The Markov chain Monte Carlo (MCMC) analysis was run for each dataset for 30x10^6 generations, sampled every 3.000 steps with the first 10% of samples discarded as burn-in

Statistical analysis for simple comparisons of the relevant distributions across different levels of categorical variables was based on Pearson’s chi-square tests as implemented in STATA 12

Results

The distributions of transmission risk groups were similar for subtypes A and B for both E138A and K103N (Table 1)

Specifically:

1. Men who have sex with men (MSM) represented 69% (N=124) and 63% (N=43) of infections with E138A in subtypes A and B, respectively (Table 1)

2. Similarly, MSM comprised 68% (N=38) and 61% (N=11) of individuals with K103N in subtypes A and B, respectively (Table 1)

Acknowledgements:
The study was in part supported by the Hellenic Society for the study of AIDS and STDs

Table 2. Characteristics for the NNRTI-resistance mutations from different subtypes

<table>
<thead>
<tr>
<th>Subtype</th>
<th>NNRTI-resistance mutation</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>E138A</td>
<td></td>
<td>1992.0</td>
<td>1987.6-1995.6</td>
</tr>
</tbody>
</table>

Figure

Bayesian skyline plots estimated by BEASTv1.8 using birth-death models (BDM) presenting the number of lineages (transmissions) over time for the NNRTI-resistance mutations (E138A and K103N) from different subtypes (A and B)

Discussion

This is one of the few studies highlighting differences in transmission dynamics of resistant strains belonging to different subtypes

Specifically, our study suggests that E138A and K103N resistant mutations are transmitted at higher rates in subtype A than in subtype B strains

Acknowledgements:
The study was in part supported by the Hellenic Society for the study of AIDS and STDs

References:

1. Men who have sex with men (MSM)
2. Women who have sex with women (MSW)
3. Men who have sex with others (MSW)
4. People who inject drugs (PWID)

Table 1. Distribution of transmission risk groups for the NNRTI-resistance mutations from different subtypes

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Transmission risk group</th>
</tr>
</thead>
<tbody>
<tr>
<td>E138A</td>
<td></td>
</tr>
<tr>
<td>K103N</td>
<td></td>
</tr>
</tbody>
</table>

*Contact Information: dparask@med.uoa.gr
Background

Previous studies on pre-treatment drug resistance from sub-Saharan Africa have shown the highest prevalence in Uganda, particularly in Kampala, with a prevalence of 12.3%. Antiretroviral therapy (ART) has been publicly available in Uganda since 2000, with initial use - although limited - of mono/dual thymidine analogues. This study aims to describe type and frequency of pre-treatment resistance in HIV-infected Ugandan adults seeking care at one of the largest public-sector providers in Kampala, Uganda.

Methods

From June 4th – September 30th, 2015, ART-naïve adults (≥18 years) presenting to the Infectious Diseases Institute (IDI) in Kampala and willing to participate in this study, were asked to give a plasma sample for pre-treatment HIV genotyping. Sequencing of partial polymerase gene was conducted using an in-house protocol. All sequences were submitted to the Stanford University HIV Drug Resistance database and the surveillance drug resistance mutations were identified using the 2009 WHO mutations list.

Results

Pre-treatment drug resistance testing was available from 152 ART-naïve HIV-infected adults, of which 96 (63.2%) were female with a median age of 33 years (interquartile range (IQR) 26-41), and a median CD4 cell count of 511 cells/uL (IQR 284-713). Mutations associated with HIV drug resistance were found in 9/152 (5.9%) patients. Five patients (5/152, 3.3%) harbored NRTI mutations, and 8/152 (5.3%) had NNRTI mutations. Five (3.3%) patients had one class mutations, and 4 (2.6%) showed double class resistance. Protease inhibitor mutations were not observed (for specific mutations see table).

<table>
<thead>
<tr>
<th>Drug class / mutations</th>
<th>Total N = 152, (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any NRTI mutation</td>
<td></td>
</tr>
<tr>
<td>K65R</td>
<td>5 (3.3)</td>
</tr>
<tr>
<td>M184V</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Other (M41L, T215I)</td>
<td>2 (1.3)</td>
</tr>
<tr>
<td>Any NNRTI mutation</td>
<td></td>
</tr>
<tr>
<td>K101E</td>
<td>3 (2.0)</td>
</tr>
<tr>
<td>Y181C</td>
<td>2 (1.3)</td>
</tr>
<tr>
<td>K103N</td>
<td>2 (1.3)</td>
</tr>
<tr>
<td>Other (M230L, G190A/S, Y188L)</td>
<td>4 (2.6)</td>
</tr>
</tbody>
</table>

Conclusions

Contrary to previous reports, we found a low prevalence of pre-treatment drug resistance among Ugandan adults in Kampala. We hypothesize that the use of mono/dual thymidine analogues in the past contributed to a higher circulation of TAMs, as observed in developed settings. The subsequent swift scale-up of triple ART in the region may have reduced pre-treatment resistance over time.

Acknowledgements: We would like to acknowledge all patients and their families.

Funding: Swiss HIV Cohort Study, Gilead Sciences
Moderate prevalence of rilpivirine resistance in people starting antiretroviral treatment in Argentina

E. Bissio, M. G. Barbás, S. Kademián, M. B. Bouzas, H. Salomón, A. Cudolá, C. Falistocco

Background: Rilpivirine-based regimens are now preferred or alternative first-line regimens according to many treatment guidelines. In low- and middle-income countries (LMIC), efavirenz-based regimens continue to be the recommended choice for ART initiation. Due to its relative low cost and better tolerance, rilpivirine might become an alternative for ART initiation in some LMIC. Recently, a surveillance study performed in Argentina determined that prevalence of resistance to 1st generation NNRTIs among people starting ART without previous exposure was 10%. However, resistance to newer-generation NNRTIs was not analyzed since these are not yet recommended as first-line therapy in this country. The aim of this study was to analyze the prevalence of resistance mutations to newer generation NNRTIs in the population starting ART in Argentina.

Methods: We analysed the prevalence of resistance mutations to rilpivirine and etravirine (according to the IAS list), obtained through a nationally representative pretreatment HIV-drug resistance (PDR) surveillance study performed in Argentina in 2014-2015. Briefly, 25 ART-dispensing sites throughout the country were randomly chosen to enrol 330 adults starting ART; to generate a point prevalence estimate of resistance-associated mutations (RAMs) with a maximum 5% confidence interval. Samples were processed with Trugene (Siemens), and analysed using the Stanford algorithm. Mutations conferring resistance to second-generation NNRTIs were not considered in the original analysis. For the present analysis, we incorporated the mutations associated with resistance to rilpivirine and etravirine, according to the last version of the IAS list. The Stanford HIVdb algorithm was used to classify HIVDR. Sequences classified as “susceptible” and “potential low-level resistance” were considered as having no resistance.

Results: Participants were recruited from 25 survey sites (accounting for 30). We enrolled 330 participants from whom we collected plasma specimens for resistance testing. For the present analysis, only those without prior antiretroviral exposure were considered (n=270). Mean (fifSD) age was 36.7 years (IQR 31-59); 66.7% were male, 0.7% were transgender; median (IQR) CD4 cell count was 284/mm3 (132-488); median (IQR) viral load: 56993 copies/ml (7534-119000); time on ART was 2.2 years (0.3-2.5); however, 60% of study participants were diagnosed within 6 months prior to study entry.

Regarding viral subtype distribution, 46% were subtype B, 46% were subtype BF, 4% were subtype C, 2% subtype F and 2% the viral subtype could not be determined.

The prevalence (95% CI) of RAMs to any antiretroviral (including newer generation NNRTIs) was 16% (11-20). The prevalence (95% CI) of resistance to NNRTIs was 13% (9-17). Regarding resistance to rilpivirine, 19/239 samples harboured any RAM to this antiretroviral, translating into a prevalence (95% CI) of RAMs to etravirine was 4% (2-7).

Conclusions: This PDR surveillance study showed concerning levels of HIVDR in Argentina, not only for first-generation NNRTIs but also to rilpivirine and, to a lesser extent, etravirine. In our setting, performing resistance testing would be necessary before prescription of ART even if the person is prescribed a second-generation NNRTI based regimen.

Table

<table>
<thead>
<tr>
<th></th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of samples with NO prior exposure</td>
<td>270</td>
</tr>
<tr>
<td>Samples successfully sequenced</td>
<td>269</td>
</tr>
<tr>
<td>HIVDR prevalence (95% CI)</td>
<td>19/239 (8% (5-11))</td>
</tr>
<tr>
<td>NNRTI RAMs prevalence (95% CI)</td>
<td>30/239 (13% (8-17))</td>
</tr>
<tr>
<td>RILPIVIRINE RAMs prevalence (95% CI)</td>
<td>19/239 (8% (5-11))</td>
</tr>
<tr>
<td>ETRAVIRINE RAMs prevalence (95% CI)</td>
<td>10/239 (4% (2-7))</td>
</tr>
</tbody>
</table>

References:
Prevalence of HIV type 1 drug resistance mutations in
treatment-naïve patients participating in the GARDEL Study

Maria Inés Figueroa, Patricia Patterson, Pedro Cahn, Jaime Andrade-Villanueva, José R Arribas, José M Gatell, Javier R Lama, Michael Norton, Juan Sierra Madero, Omar Sued, Maria José Rolón, on behalf of the GARDEL Study Group*

BACKGROUND
Combination antiretroviral therapy has greatly reduced the rate of morbidity and mortality among HIV-1 infected patients. However, high mutation and recombination rates of HIV-1 lead to the emergence of various subtypes and drug-resistance viruses, rendering first line ARV-therapy ineffective in many patients.

The aim of this sub study is to describe the prevalence of HIV-1 subtypes and the patterns of drug resistance mutations among ARV-naïve HIV-1-infected patients from six different countries participating in the GARDEL Study.

MATERIALS AND METHODS
543 naïve patients from 6 countries (Argentina, Chile, Spain, Mexico, Peru and US) were screened between Dec-2010 to May 2012, and 534 HIV-sequences were analyzed following the IAS-USA 2014 Drug Resistance Mutations Panel. Genotypic assays performed at screening visit were: PhenoSense HIV assay (Monogram Biosciences, San Francisco, CA, USA), ViroSeq HIV-1 (ViroSeq HIV-1 Genotyping System v2.0; Celera, Alameda, CA), TRUGENE® HIV-1 Genotyping Assay (Siemens Healthcare Diagnostics), according to availability at each site.

RESULTS
Of the 534 patients screened, 74% were Hispanic/Latino. Median time of infection at SCR was: 10.5 months. CDC stage A: 82%. Of 450 viral subtypes available, the most frequent was subtype B in all three regions (Fig 1). A total of 113 samples (21.2%) had major resistant mutations; 22 samples (4.1%) had major protease mutations (M46I was the most common mutation: 1.5%), 85 samples (15.9 %) had NNRTIs mutations (K103N/S was the most common mutation: 4.9%), and 17 samples had mutations to NRTIs (3.2%), M41L (1.3%) was the most common mutation to PIs, only 2 patients had only one major mutation (2/22)(Fig 2). The more frequent minor mutations were: M36I/L/V (216/534), L63P (120/534), L101I/F/V/R (115/534) and K20R/M/I (59/534). The global resistance analysis by regions showed 21% for LA, 22.8% for US/Mexico and 14.7% for Spain, being NNRTI resistance by regions 16.4%; 15.4% and 11.8% respectively. PI resistance was 3.1% for LA and Mexico/US and NRTI resistance was 3.1% for LA, 3.4% for US/Mexico and 2.9% for Spain. No Q151M, 69ss or K65R were identified.(Fig 3)

CONCLUSIONS
In our study we found a primary resistance rate of 21.2%, similar in LA and US/Mexico but lower in Spain. Levels of NNRTI resistance are similar in the three analyzed regions, as previously reported in naïve populations, and reinforces the need of performing genotypic testing in ARV naïve patients, especially in LA were the first line therapy is still based on NNRTI drugs.

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Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are particularly prone to treatment failure as high-level drug resistance has been associated with a single point mutations within the binding site of reverse transcriptase. Thus, transmitted NNRTI mutations, may contribute to an increased risk of virologic failure for patients prescribed their first ART regimen.

Our study investigated the NNRTI resistance profiles of antiretroviral-naïve patients in a large urban clinic setting (Maple Leaf Medical Clinic, Toronto, ON) and assessed their response to their initial antiretroviral therapy (ART).

The three objectives of this study are:
1. To assess the frequency of NNRTI, NRTI and PI mutations
2. To report if the frequency of baseline NNRTI mutations affects time to virologic suppression
3. To report if the frequency of baseline NNRTI mutation affects time to virologic rebound in those who have achieved virologic suppression

Methods

This was a retrospective clinical chart review of ART-naïve patients with available baseline genotypes whom were prescribed their first ARV regimen.

Inclusion criteria:
1. HIV-positive
2. Aged 16 years or older at baseline
3. Has a baseline genotype between January 1, 1997 and July 16, 2015 prior to starting ART

Statistical Analysis:
For demographic and clinical data, categorical variables were summarized using frequencies and proportions and compared using the Chi-square (Fisher) test. Continuous variables were summarized using medians and interquartile range and compared using the Wilcoxon rank sum test. Baseline NNRTI, NRTI, and PI resistance mutations were reported (Table 2) using frequencies and proportions.

• Cox regression was used to determine correlates of virologic suppression (defined as viral load (VL) < 40 (or <50 depending on era) by 6 months) with presence of baseline NNRTI resistance as the primary correlate.
• Of those with virologic suppression, we conducted Cox regression to determine correlates of virologic rebound (defined as VL ≥ 200 copies/mL).
• Censoring occurred for those who did not have any follow-up VL results and at last VL or visit date for those without evidence of viral suppression.

RESULTS

Baseline demographic are shown in Table 1. Of the 1338 patients with a baseline genotype, we further looked at the 1218 that subsequently initiated ARV's.

When treated, patients without baseline NNRTI mutations (n = 1135) were prescribed NNRTI-containing regimens in 43.9% of cases, PI-containing regimens in 34.7% of cases and INI-containing regimens in 14.3% of cases. Treated patients with baseline NNRTI resistance (n = 83) were prescribed PI-containing regimens in 51.8% of cases and INI-containing regimens in 28.9% of cases. Baseline mutation frequencies by class are shown in Table 2.

Virologic suppression was observed in 1024 out of 1218 (84.07%) individuals whom were prescribed ARVs. 83.13% of patients with baseline NNRTI mutation achieved viral suppression while 84.14% without NNRTI mutations achieved suppression (Table 3).

• In univariate and multivariate Cox regression, the presence of baseline NNRTI resistance did not impact virologic suppression (HR = 0.98; 95%CI = 0.76-1.24).
• For virologic rebound, the presence of baseline NNRTI resistance also did not impact its occurrence (HR = 1.11; 95%CI = 0.68-1.81).
• In multivariable analysis, after adjusting for age, gender, baseline VL and CD4 count, duration of HIV and baseline PI mutations, the presence of NNRTI mutations also did not impact virologic rebound (aHR = 1.09; 95%CI = 0.66-1.78) (Table 4).

CONCLUSIONS
• Baseline NNRTI mutations were present in 6.7% of our antiretroviral-naïve patients.
• Despite having baseline NNRTI mutations, the majority of patients (83.13%) reached virologic suppression and did not experience increased risk virologic rebound.
• Few new mutations were developed in those who started ART.
• Patients with NNRTI mutations are being treated effectively with increased use of other ARV classes.
Enhanced surveillance to study HIV-1 drug resistance among naive individuals in Greece: the added value of molecular epidemiology to public health


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Introduction

HIV-1 transmitted drug resistance (TDR) to NNRTIs has been shown to compromise first-line response to treatment. The prevalence of resistance to NNRTIs was previously estimated to be 16.9% among drug naive individuals in Greece

Aim

Our aim was to investigate the dispersal patterns of HIV-1 resistant strains and to estimate the effective reproductive number (Re) and transmission dynamics for locally transmitted resistance.

Materials and Methods

We analyzed sequences from 3,428 HIV-1 treatment naive individuals available in the PRTIR. Sequences were sampled in Southern Greece during 01/01/2003 - 31/06/2015

Phylogenetic analysis was performed on subtype A (N=235) and B (N=86) sequences with resistance to NNRTIs (K103N and E138A) (Table 1 and 2), along with sequences isolated from seropositives without resistance from Greece sampled during 1998 - 2013 (subtype A: N=904; subtype B: N=1,615) and a randomly selected global dataset (subtype A: N=5,907; subtype B: N=3,984). Phylogenetic trees were inferred by maximum likelihood (ML) method as implemented in RAxML v8.0.20

Phylogenetic analyses revealed that:

- For subtype A the majority of individuals infected with resistant strains (209 out of 235, 88.9%) belonged to monophyletic clusters (local transmission networks, LTNs) (Figure 1 A). Specifically, 48 out of 56 (85.7%) of sequences with K103N, and 148 out of 179 (82.7%) with E138A belonged to one and four LNTs, respectively (Figure 1 A). These findings suggest that the viruses with the most prevalent resistance mutations spread locally.
- For subtype B either non-clustered sequences or small LNTs (range: 2-6 sequences), were identified (Figure 1 B)

Molecular clock analyses revealed that:

- For the K103N sub-outbreak the Re was higher than 1 between 2008 and the first half of 2013 (maximum value of median Re = 2.8) (Table 3, Figure 2). On the other hand, for all E138A LNTs the Re was higher between 2011 and 2015, except the most recent one where the Re was approximately equal to 1 (Figure 2)

Results

Phylogenetic analyses revealed that:

- For subtype A the majority of individuals infected with resistant strains (209 out of 235, 88.9%) belonged to monophyletic clusters (local transmission networks, LTNs) (Figure 1 A). Specifically, 48 out of 56 (85.7%) of sequences with K103N, and 148 out of 179 (82.7%) with E138A belonged to one and four LNTs, respectively (Figure 1 A). These findings suggest that the viruses with the most prevalent resistance mutations spread locally.
- For subtype B either non-clustered sequences or small LNTs (range: 2-6 sequences), were identified (Figure 1 B)

Molecular clock analyses revealed that:

- For the K103N sub-outbreak the Re was higher than 1 between 2008 and the first half of 2013 (maximum value of median Re = 2.8) (Table 3, Figure 2). On the other hand, for all E138A LNTs the Re was higher between 2011 and 2015, except the most recent one where the Re was approximately equal to 1 (Figure 2)

Discussion

- Our study suggests that the most prevalent mutations associated with resistance to NNRTIs were transmitted through local networks in Greece.
- Notably, phylogenetic analysis allows estimating that resistance in the last few years has been actively propagated with an increasing incidence.
- Those belonging to the active TDR networks are the priority population to intervene.

Phylogenetic analyses were performed using birth-death models (BDM) allowing estimation of important epidemiological parameters such as the effective reproductive number (Re), as implemented in BEAST2. The Re is defined as the expected secondary infections per infected individual

Table 1. Distribution of HIV-1 subtypes for NNRTI-resistance mutations

<table>
<thead>
<tr>
<th>Subtype</th>
<th>NNRTI-resistance mutation (N, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E138A</td>
<td>K103N</td>
</tr>
<tr>
<td>A</td>
<td>179 (68) 56 (70)</td>
</tr>
<tr>
<td>B</td>
<td>68 (26) 18 (23)</td>
</tr>
<tr>
<td>Other</td>
<td>16 (6)  6 (7)</td>
</tr>
</tbody>
</table>

Table 2. Distribution of transmission risk groups and sampling periods for the NNRTI-resistance mutations from different subtypes

<table>
<thead>
<tr>
<th>Subtype</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>NNRTI-resistance mutation</td>
<td>E138A K103N E138A E138A K103N</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Characteristics for the five transmission networks

<table>
<thead>
<tr>
<th>Transmission network</th>
<th>MRCA median; 95% HPD</th>
<th>Re (maximum value of median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E103N</td>
<td>2007 (2004-2009) 2.8</td>
<td></td>
</tr>
<tr>
<td>E138A_2</td>
<td>1990 (1989-2000) 1.8</td>
<td></td>
</tr>
</tbody>
</table>

*Transmission risk group (MRCA, median; 95% HPD) Re (maximum value of median)
Transmission patterns of HIV-1 subtype A resistant strains across Greece: Evidence for country and regional level transmission networks


1Department of Hygiene, Epidemiology and Medical Statistics, National and Kapodistrian University of Athens, Athens, 2Department of Microbiology, AHEPA University Hospital, Aristotle University of Thessaloniki, Thessaloniki, 31st Department of Internal Medicine, G. Genimatas GH, Athens, 43rd Internal Medicine Department-Infectious Diseases, Red Cross Hospital, Athens, 5th Department of Medicine and Infectious Diseases, Evangelismos GH, Athens, 4th Department of Medicine, Attikon GH, Medical School, National and Kapodistrian University of Athens, Athens, Laikon GH, Medical School, National and Kapodistrian University of Athens, Athens, 4th Department of Medicine, National and Kapodistrian University of Athens, Athens, 2nd Department of Internal Medicine, Sismanogleion GH, Athens, 12Department of Internal Medicine, University GH, Democritus University of Thrace, Alexandroupolis

Introduction

The prevalence of mutations conferring resistance to NNRTIs was previously reported to be higher than 15% among drug naïve individuals both in Northern and Southern Greece. The most prevalent resistance mutations were E138A, K103N and Y181C associated mostly with subtype A1.

Our aim was to investigate the dispersal patterns of HIV-1 resistant strains across Greece.

Materials and Methods

We analyzed sample of subtype A1 sequences (N=1,104) available in the pol gene (PT/RT)

Sequences were sampled in Northern and Southern Greece during 1999 and middle-2015. We included sequences only from Greece since we have shown previously that subtype A1 sequences have been mostly found within a single monophyletic cluster.

Phylogenetic analysis revealed that:

- E138A and K103N resistant strains have spread through large monophyletic clusters spanning both Northern and Southern Greece, suggesting that all transmissions within these clusters occurred regionally (Figure).
- Conversely to E138A and K103N, Y181C formed a subnetwork (monophyletic cluster) limited in Northern Greece with only a single spill over to Southern Greece (Figure).
- For K103N strains we found a large (N=49) and a small cluster (N=5) including sequences from both areas (Figure).
- Sequences from Northern Greece formed two specific subnetworks, suggesting local dispersal (Figure).
- Sequences with E138A from Northern Greece formed two specific subnetworks within the E138A monophyletic clades found for Greece. The latter consisted of four major clades of 53, 41, 29 and 25 sequences from both regions (Figure).
- Overall, E138A and K103N spread through common networks across the country with evidence of local transmissions in Northern Greece (Figure).
- On the other hand, Y181C has spread only in Northern Greece with very limited dispersal to Southern Greece (Figure).

Results

Phylogenetic topology (tree) was estimated from the underlying nucleotide sequences using approximate maximum likelihood (ML) method with bootstrapping as implemented in RAxML v8.0.20

Specifically, analysis was performed under the Generalized Time Reversible (GTR-cat) model of nucleotide substitution model including a f distributed rate of heterogeneity among sites.

Discussion

- A high prevalence of NNRTI resistance mutations was previously reported for the subtype A1 strains circulating in Greece and especially in Northern Greece.
- The majority of these resistant viruses were transmitted within common transmission networks.
- Significant clustering of sequences from Northern Greece as well as the existence of a regional cluster suggest high transmission networking of the population in this area; a finding that might explain the higher prevalence of transmitted drug resistance (TDR) in Northern Greece.
- Our study highlights the priority population to prevent TDR in the future.

Acknowledgments:
The study was in part supported by the Hellenic Society for the study of AIDS and STDs

Contact Information: dparask@med.uoa.gr
Occurrence and Risk Factors for Primary Integrase Resistance-associated Mutations in Austria in the years 2008-2013

A. Zoufaly 1, Kraft C1, Schmidbauer C1, Puchhammer-Stöckl E2

1 Department of Medicine IV, Kaiser Franz Josef Hospital, Vienna, Austria; 2Department of Virology, Medical University Vienna, Austria

Introduction:
In Europe, country specific treatment guidelines often do not advocate testing for Integrase inhibitor resistance associated mutations (IRAM) before initiation of first line ART given the extremely low prevalence of mutations found in older surveillance studies. However, increased use of integrase inhibitors (INSTI) might have led to the emergence of treatment limiting mutations in more recent years. We aimed to determine the prevalence of IRAM in Austria in the 5 years following introduction of INSTI and to analyze trends and factors associated with their detection.

Methods:
Samples of ART naïve patients in Austria between 2008 and 2013 were analyzed for the existence of IRAM using bulk sequencing with published primers and drug resistance penalty scores (Stanford HIVdb algorithm) were calculated to estimate response to antiretroviral drugs. Demographic and virological data including age, sex, viral subtype, drug resistance associated mutations to PI and RTI were extracted from a database. Comparative statistics and logistic regression models were used to analyse risk factors for the occurrence of IRAM.

Results:
A total of 303 samples were analyzed. Patient characteristics are shown in Table 1. Overall prevalence of IRAM was 2.3%.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean age (years, SD)</th>
<th>Male sex (n, %)</th>
<th>Year of sample (n, %)</th>
<th>Year of sample (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years, SD)</td>
<td>38 (12)</td>
<td>235 (77.6%)</td>
<td>2008 49 (16.2%)</td>
<td>2008 49 (16.2%)</td>
</tr>
<tr>
<td>Male sex (n, %)</td>
<td>235 (77.6%)</td>
<td></td>
<td>2009 51 (16.8%)</td>
<td>2009 51 (16.8%)</td>
</tr>
<tr>
<td>Year of sample (n, %)</td>
<td></td>
<td></td>
<td>2010 51 (16.8%)</td>
<td>2010 51 (16.8%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2013 49 (16.2%)</td>
<td>2013 49 (16.2%)</td>
</tr>
<tr>
<td>Viral load categories (HIV RNA copies/ml,n,%)</td>
<td>&lt;1x10^4</td>
<td>7 (6.7%)</td>
<td>1x10^4&lt;5x10^4</td>
<td>23 (21.9%)</td>
</tr>
<tr>
<td>Viral subtype (n, %)</td>
<td></td>
<td></td>
<td>A 22 (7.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B 188 (62.1%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C 35 (11.6%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>other 58 (19.1%)</td>
<td></td>
</tr>
<tr>
<td>Major/primary drug resistance present (n, %)</td>
<td>NRTI 4 (1.3%)</td>
<td>NNRTI 20 (6.6%)</td>
<td>PI 5 (1.7%)</td>
<td>INSTI 1 (0.3%)</td>
</tr>
</tbody>
</table>

Table 1: Patient Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male sex (n, %)</th>
<th>Year of sample (n, %)</th>
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</tr>
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<tbody>
<tr>
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</tr>
<tr>
<td>Viral subtype (n, %)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B 188 (62.1%)</td>
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<td></td>
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<tr>
<td></td>
<td>C 35 (11.6%)</td>
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<tr>
<td></td>
<td>other 58 (19.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major/primary drug resistance present (n, %)</td>
<td>NRTI 4 (1.3%)</td>
<td>NNRTI 20 (6.6%)</td>
<td>PI 5 (1.7%)</td>
</tr>
</tbody>
</table>

Table 2: Drug penalty scores (Stanford HIVdb algorithm) indicating susceptibility to Integrase inhibitors

<table>
<thead>
<tr>
<th>Year</th>
<th>Raltegravir (n)</th>
<th>Elvitegravir (n)</th>
<th>Dolutegravir (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>47</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2009</td>
<td>47</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2010</td>
<td>50</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2011</td>
<td>50</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2012</td>
<td>50</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2013</td>
<td>50</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3: Patients with Integrase resistance associated mutations

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>0.80</td>
<td>0.36</td>
<td>0.58</td>
</tr>
<tr>
<td>Age (per year)</td>
<td>0.98</td>
<td>0.96</td>
<td>1.01</td>
</tr>
<tr>
<td>PI/NNRTI or NNRTI mutation</td>
<td>0.44</td>
<td>0.10</td>
<td>1.96</td>
</tr>
<tr>
<td>Calendar year</td>
<td>1.04</td>
<td>0.86</td>
<td>1.27</td>
</tr>
<tr>
<td>Subtype B virus</td>
<td>0.54</td>
<td>0.06</td>
<td>4.60</td>
</tr>
</tbody>
</table>

Table 4: Risk factors for Integrase resistance associated mutations

Conclusions:
- Major primary IRAM are rarely found despite increasing use of INSTI in Austria
- There is potential for reduced susceptibility to these drugs in selected patients
- No clear risk factors for occurrence of IRAM can be identified
- Routine resistance testing seems prudent to avoid the consequences including accumulation of further mutations and therapeutic failure

References
1) Steklar JD et al, Antivir Ther. 2015;20(1):77-80
2) Salataini F et al, Clin Microbiol Infect. 2012;18(10)
4) DAIG, Deutsch-Österreichische Leitlinien. 2015

Acknowledgement and financial disclosure
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Transmission of HIV-1 Drug Resistance in Tel-Aviv, Israel, 2010-2015

Dan Turner*, Shirley Girshengorn, Adi Braun, Luba Tau, Ari Leshno, Danny Alon, Tal Pupko, Irena Zejdls, Svetlana Ahsanov, Simona Gielman, Natasha Matus, Inbal Schweitzer, and Boaz Avidor

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2Department of Cell Research and Immunology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat Aviv 69978, Israel
3Laboratory for Viruses and Molecular Biology, Tel Aviv Sourasky Medical Center, Tel-Aviv, Israel.

Background: As of 2010, testing for HIV drug resistance is performed routinely to all new HIV patients followed-up in Tel-Aviv. Thus, the objective of this study was to evaluate transmission drug resistance mutations (TDR) among HIV-1 treatment-naïve patients in Tel-Aviv from 2010 to 2015.

Methods: The first blood samples obtained between 2010 and 2015 from each treatment-naïve patients joining Tel Aviv HIV clinic after the diagnosis of HIV were sequenced for protease and reverse transcriptase (RT) regions. TDR in these two regions were defined according to the criteria proposed by Bennett et al. [2009]. PLoS ONE 4:e4724. Subtyping of the isolates was based on the Stanford HIV Drug Resistance Database. Phylogenetic reconstruction was inferred using pol sequences. Multiple sequence alignments were computed using MAFFT v. 7.7. The phylogenetic tree was then inferred using maximum likelihood as implemented in PHYLYP. Likelihood computations were based on the HKY model, taking among site rate variation into account (i.e., the gamma parameter). Confidence in tree estimation was based on 100 bootstrap replications. Ethical approval for the study was granted by the institutional ethics committee.

Results: Table 1 shows the characteristic of the patients. MSM was the major exposure risk category (ERC) group followed in Tel-Aviv, 76 % among them were born in Israel, and 83 % harbor subtype B viruses. Other groups include intravenous drug users (IVU): 78 % of them were born in the former Soviet Union countries and 86% harbor subtypes A viruses. The heterosexual group is very heterogeneous and includes patients born in Israel, Ethiopian immigrants, immigrants from the former Soviet Union, and worker immigrants mainly from Africa. Rate of TDR is described in tables 2 and 3. The resistance rate decreased from 15.1% in 2010 to 6% in 2013 (P < 0.05). In 2014 and 2015 we noted an increase to 13.9% and 14.4% respectively. In 2010-2011 protease inhibitors (PIs) was the major resistance mutation, while in 2014-2015 NRTIs resistance mutation was dominant.

Phylogenetic analysis of subtypes A, B and C was performed on 610 sequences (Fig. 1). In subtype A viruses we found a cluster among IVU at 2012 during an outbreak, without resistance associated mutation. However, a cluster with viruses harboring resistance mutation at position 103 was found in five MSM and one IVU female. The analysis subtype B viruses support clustered TDR among MSM. Among subtype C viruses there were no specific clusters.

Discussion: TDRs among patients followed in Tel-Aviv were represented by clusters in MSM. These clusters contain resistance associated mutations to drugs less prescribed in recent years. Although the region of the integrase gene is not routinely sequenced in treatment-naïve patients followed-up in our center, low rate of Inl TDR is reported in other studies.
Development of T66I-mediated integrase inhibitor cross-resistance against elvitegravir under dolutegravir containing firstline therapy

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2 Medical Center for Internal Medicine and Rheumatology, Freiburg, Germany

Background

As second generation integrase inhibitor (INI), dolutegravir (DTG) has shown a superior barrier to resistance as compared to profiles of raltegravir (RAL) or elvitegravir (EVG). Current findings suggest that resistance mutations against INIs (Fig.1) extreme rarely occur under DTG-containing first line antiretroviral therapy (ART)*. This case report unveils a possible development of a T66I-mediated cross-resistance against EVG under a DTG firstline regimen (Tab.1).

Methods

A firstline treatment with lamivudin/abacavir, lopinavir and dolutegravir was initiated by a 44 years old man with a diagnosis of HIV in 11/2015 (CDC status: B2, CD4 nadir: 219/µl, HIV-1 RNA: 350,000 copies/mL). Ultra-deep sequencing was performed by using population sequencing and ultra-deep sequencing (UDS, Illumina MiSeq) at baseline and at time of therapy failure. Resistance interpretation was estimated by using the HIV-Grade 12/2015, Stanford HIV-db 7.0.1, Rega 9.1.0 and the ANRS 25_09/2015 database. Viral load was quantified with Abbott Realtime.

Results

Before start of therapy, no resistance-associated variants could be detected neither by population or by UDS in HIV protease, reverse transcriptase and integrase (Table 2). After start of DTG-firstline therapy, HIV viral load dropped from 302,815 copies/ml to 2,400 copies/ml within four weeks of follow up and was undetectable at week 8. CD4 cell counts increased from 219/µl to 479/µl (13.4%). However, 20 weeks after initiation of ART, HIV viral load increased to 105 copies/ml and maintained low viremic four weeks later at 112 copies/ml most likely due to inadequate adherence although plasma drug levels turned out to be above critical limits.

More importantly, the development of the INI-resistance mutation T66I was then verified by UDS showing a minority population of 36.1%. The variant T66I is a non-polymorphic mutation and reduces EVG susceptibility by ~15-fold while susceptibility to RAL or DTG is reported to be unaffected. There was no evidence for protease or reverse transcriptase resistance mutations at this time. 28 weeks after initiation of ART, HIV viral load decreased to undetectable levels without any changes.

Conclusions

Although being extreme rarely observed, INI-resistant HIV variants may also occur under DTG firstline treatment. The T66I alone does not necessarily limit the susceptibility to DTG itself but could be a first step of resistance development against DTG. It is reported that T66I confer high-level resistance against EVG and may also putatively lower the resistance barrier against RAL.

<table>
<thead>
<tr>
<th>Year of analysis</th>
<th>T66 variant</th>
<th>RT/TP resistance*</th>
<th>ART</th>
<th>Subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>T66A</td>
<td>Yes</td>
<td>LPV/r; RAL</td>
<td>B</td>
</tr>
<tr>
<td>2015</td>
<td>T66A</td>
<td>No</td>
<td>Naive</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>T66K</td>
<td>Yes</td>
<td>TDF/FTC/COB/ EVG</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>T66I</td>
<td>Yes</td>
<td>TDF/FTC/ DRV/r</td>
<td>CRF02_AG</td>
</tr>
<tr>
<td></td>
<td>T66I</td>
<td>Yes</td>
<td>TDF/FTC/ COB/ EVG</td>
<td>B</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>11/2015</th>
<th>12/2015</th>
<th>01/2016</th>
<th>04/2016</th>
<th>05/2016</th>
<th>06/2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>ART</td>
<td>3TC/ABC; LPV/r, DTG</td>
<td>3TC/ABC; LPV/r, DTG</td>
<td>3TC/ABC; LPV/r, DTG</td>
<td>3TC/ABC; LPV/r, DTG</td>
<td>3TC/ABC; LPV/r, DTG</td>
<td>3TC/ABC; LPV/r, DTG</td>
</tr>
<tr>
<td>DTG TDM (opt. ~500µg/ml)</td>
<td>---</td>
<td>1599ng/ml (18 h)</td>
<td>---</td>
<td>---</td>
<td>2619ng/ml (10 h)</td>
<td>---</td>
</tr>
<tr>
<td>Viral load</td>
<td>303,815</td>
<td>2,430</td>
<td>0</td>
<td>105</td>
<td>112</td>
<td>0</td>
</tr>
<tr>
<td>CD4 (abs.)</td>
<td>219</td>
<td>313</td>
<td>479</td>
<td>427</td>
<td>485</td>
<td>587</td>
</tr>
<tr>
<td>Resistance (Pop.-Seq) No RAMs</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>T66I/T</td>
<td>---</td>
</tr>
<tr>
<td>Resistance (UDS) No RAMs</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>T66I (36,5%)</td>
<td>---</td>
</tr>
</tbody>
</table>
Patterns of emergent resistance-associated mutations after initiation of non-nucleoside reverse-transcriptase inhibitor-containing regimens in Taiwan: a multicenter cohort study

Chien-Yu Cheng¹, Yi-Ching Su², Wen-Chun Liu², Shu-Hsing Cheng³, Hsin-Yun Sun³, Chien-Ching Hung³, Sui-Yuan Chang³

¹Department of Internal Medicine, Taoyuan General Hospital, Ministry of Health and Welfare, Taoyuan, Taiwan
²Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan
³Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan

Background
Non-nucleoside reverse-transcriptase inhibitor (NNRTI)-containing antiretroviral therapy (ART) remains the recommended first-line regimens for adults infected with HIV in many resource-limited countries. Increasing trends of resistance-associated mutations (RAMs) to nNRTIs have caused concerns about the effectiveness of the regimens in national programs in these regions [1-3]. In this multicenter study, we aimed to investigate the incidence of emergent RAMs of HIV-1 to antiretrovirals (ARVs) in HIV-positive adults who developed virological failure to first-line nNRTI-containing ART in Taiwan.

Methods
Between June 2012 and March 2016, ARV-naïve HIV-positive adults who initiated 2 NRTIs plus NNRTI at participating hospitals were included for analysis. Plasma HIV RNA load (PVL) was determined at baseline, and week 4-6 and subsequently every 12 to 16 weeks after ART initiation. Virological failure was defined as a decrease of PVL < 1.0 log₁₀ copies/ml in 4 to 6 weeks of ART initiation; or PVL ≥ 200 copies/ml at 6 months of ART initiation; or confirmed HIV RNA ≥ 200 copies/ml after viral suppression (PVL < 50 copies/ml). Population sequencing was used to detect RAMs. Detection of RAMs at baseline was performed retrospectively. RAMs were interpreted using the IAS-USA 2015 mutations list.

Figure 1. Prevalence of emergent resistance-associated mutations among three different non-nucleoside reverse transcriptase inhibitor-containing regimens.

![Prevalence of emergent resistance-associated mutations among three different non-nucleoside reverse transcriptase inhibitor-containing regimens.](image)

Results
During the 3.5-year study period, 1642 patients initiated nNRTI-containing regimens, and 454 (27.4%) had to switch first-line ART because of adverse effects or intolerance (n=323, 19.7%), retrospective detection of RAMs at baseline (41, 2.5%), and virological failure (83, 5.1%). Virological failure to 2 NRTIs plus nevirapine, efavirenz, and rilpivirine with baseline PVL < 5 log₁₀ was 4.9% (12/245), 1.9% (11/573), and 0.7% (2/277); virological failure to 2 NRTIs plus nevirapine and efavirenz with baseline PVL > 5 log₁₀ was 16.4% (29/177) and 7.8% (29/373) respectively (Figure 1). In 68 patients (3.8%) emergent RAMs were identified: 42 patients (62.7%) with NRTI RAMs; 28 (41.2%), 1 (1.5%) and 48 patients (71.6%) with nNRTI, protease inhibitors (PI), and any ARV RAMs, respectively, and 21 (31.3%) with resistance to 2 or more classes of ARV. The common emergent RAMs to NRTIs were K65R (25%), M184I (10.3%), and M184V (36.8%), and RAMs to nNRTIs included V90I (5.9%), K101E (5.9%), K103N (19.1%), V108I (7.4%), Y181C (11.8%), and G190A (5.9%) (Figure 2).

Conclusions
While a substantial proportion of the patients discontinued first-line NNRTI-containing regimens due to adverse effects, virological response to nNRTI-containing regimens remained good in patients who were able to tolerate the regimens in Taiwan. Most common RAMs in those with virological failure were related to exposure to tenofovir disoproxil fumarate, lamivudine, nevirapine, and efavirenz.

References
Association of therapeutic failure to low level viremia in HIV-1 infected patients in the AREVIR/RESINA cohort in Germany

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BACKGROUND

- LLV has been previously associated to virological failure (VF) [1, 2]
- Therapeutic success: reduction of the HIV-1 viral load (VL) below 50 copies/ml (German-Austrian guidelines for the treatment of HIV infection)
- Low level viremia (LLV): repeated VL measurements between 50 and 200 copies/ml after initial therapeutic success

OBJECTIVES

- independent analysis of the association of LLV and other factors with VF

MATERIAL & METHODS

- AREVIR/RESINA database: clinical and virological data of therapy-naive (TN) and therapy-experienced (TE) HIV-1-infected patients in North Rhine-Westphalia, Germany
- Query of the database:
  - 2,485 first line and 3657 further-line therapies
  - Patients who attained confirmed therapeutic success under ART and experienced confirmed LLV thereafter
  - Therapies in which the VL was measured at least once every 24 weeks
- VF: confirmed viral load greater than 200 copies/ml following therapeutic success
- p-values were calculated with Fishers’ exact and Wilcoxon rank sum test

RESULTS I

- LLV occurred in 294/6142 documented therapies (4.8%) (Figure 1)
  - First-line: 47/2485 (1.9%)
  - Further-line: 247/3657 (6.8%)
  - Mean time to LLV: 27 months (σ=20.7)
  - No significant differences between first- or further line treatment (p=0.46)
- VF occurred in 56/294 (19%) cases subsequent to LLV (Figure 1)
  - Median viral load at failure: 472 copies/ml (range 203-116590 copies/ml)
  - Mean LLV episode: 77.4 weeks (σ=68.0)
- VF rate increased in TE patients (19.4%) versus TN patients (10.6%) (Figure 1)

RESULTS II

- Most risk of low level viremia (Figure 2):
  - PI-based therapies: 165/294 (56.1%)
  - NNRTI-based therapies: 76/294 (25.9%)
- Comparable VF rates of NRTI-, NNRTI- and PI-based therapies (p=20%, range 17.1-22.2%) (Figure 2)
- VF was never related to entry inhibitors or integrase inhibitors
- No risk of VF subsequent to LLV with drugs approved ≥ 2005 (p<0.001) (Figures 3 and 4)

SUMMARY & CONCLUSION

- Low level viremia (LLV) is a frequent event in the AREVIR/RESINA cohort in Germany
- VF rate increased in TE patients compared to TN patients
- Strongest predictor for VF subsequent to LLV was a treatment regimen containing drugs approved before 2005
- Episodes of LLV in patients treated with drugs with high potency and a high barrier to resistance are not predictive to VF

LITERATURE

Drug Resistance Mutations (DRM) among Pregnant HIV-Positive Women in the Duesseldorf University Hospital, Germany, 2009-2016

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Background:
Combination antiretroviral Therapy (cART) has resulted in significant reduction of mother-to-child-transmission (MTCT) from 40% to 1-2% in the last two decades. Choosing an individualized cART is one key factor for successful suppression of viral load until delivery. Thus, drug resistance testing during pregnancy before cART initiation or in case of increasing viral load is recommended. The Prevalence of DRM in pregnant women in Germany hasn’t been characterised yet.

Materials and Methods:
From 01/2009 to 03/2016 HIV-Drug-Resistance was observed in HIV-positive pregnant Women in our special consultation for pregnancies in the Department of Gastroenterology, Hepatology and Infectious Diseases in the Duesseldorf University Outpatient Clinic. The Genotypic Resistance Testing was done concerning German-Austrian pregnancy guidelines either during their first visit when they were treatment naive or being already on cART with detectable HIV-Viral Load.

Resistance Testing was performed by using Sanger Sequencing and Next generation Sequencing (NGS) by means of Illumina MiSeq-technology. Resistance Interpretation was performed by the HIV-Grade HIV-1-Tool (www.hiv-grade.de)

Results:
Data of 85 HIV-positive pregnant women and 103 live births were analysed.

The majority (88%) of these women were migrants, 75% (64/85) from Subsahara Africa, 12% (10/85) South-East-Europe, 12% (10/85) from Germany and 1% (1/85) from Asia. In 34% (29/85) they had their HIV diagnosis in their first pregnancy, in 66% (56/85) the diagnosis was upraised independently from their first pregnancy.

In 64/85 cases (75%) resistance testing was requested, with 61/64 (95%) being positive. The majority of these women were migrants, 75% (64/85) from Subsahara Africa, 12% (10/85) South-East-Europe, 12% (10/85) from Germany and 1% (1/85) from Asia. In 34% (29/85) they had their HIV diagnosis in their first pregnancy, in 66% (56/85) the diagnosis was upraised independently from their first pregnancy.

In 64/85 cases (75%) resistance testing was requested, with 61/64 (95%) being positive. The majority of these women were migrants, 75% (64/85) from Subsahara Africa, 12% (10/85) South-East-Europe, 12% (10/85) from Germany and 1% (1/85) from Asia. In 34% (29/85) they had their HIV diagnosis in their first pregnancy, in 66% (56/85) the diagnosis was upraised independently from their first pregnancy.

Most common mutations were: M184VI (5/14), T215Y/F/N (4/14), Y181C (3/14) and K103N (3/14).

NGS-analysis showed additional mutations in 2/14 patients in comparison to Sanger: in Patient No. 1 (T215YF/N) in Patient No. 8 (M230I) to Sanger DRM.

In 2/14 Therapy-naïve patients tDRM could be shown only in NGS-sequencing : the revertant T215N in patient No. 12 and the K65R in patient No. 2.

No case of MTCT has been observed.

Conclusions:
In 23% (14/61) of all HIV-positive pregnant women in our study DRM have been observed, in 8% tDRM (5/61). The prevalence of tDRM in pregnant women in our population is lower than in general German population of HIV-positive individuals [2].

Using resistance testing by NGS resulted in the identification of additional relevant DRM compared to Sanger. Considering the importance of viral load suppression in Pregnancy and the limited amount of time to achieve this goal, the choice of cART should be optimal and take these mutations into account. Especially women from Subshara Africa harbour the risk of tDRM because of the CART regimens in High prevalence countries. The number of drug Resistance testing in developing countries is increasing.

Genotypic Resistance Testing should be therefore considered for all pregnant women to optimize the success of cART and hence prevent mother to child transmission.

References:
1. Noguera-Julian M, Cozzi-Leprini A., di Giannolando F et al. ; CROI 2014; Poster 600

Figure 1: Origin of n=85 HIV-positive pregnant women

Table 1: VL=HIV Viral Load, HIV-1 Subtypes, Resistance Testing by Sanger Sequencing and Next generation Sequencing (NGS), PR= Protease Mutations, RT= Reverse Transcriptase mutations, ART History

<table>
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Prevalence of HIV type 1 drug resistance mutations in treatment-naïve patients participating in the GARDEL Study

Maria Inés Figueroa, Patricia Patterson, Pedro Cahn, Jaime Andrade-Villanueva, José R Arribas, José M Gatell, Javier R Lama, Michael Norton, Juan Sierra Madero, Omar Sued, Maria José Rolón, on behalf of the GARDEL Study Group*

BACKGROUND

Combination antiretroviral therapy has greatly reduced the rate of morbidity and mortality among HIV-1 infected patients. However, high mutation and recombination rates of HIV-1 lead to the emergence of various subtypes and drug-resistance viruses, rendering first line ARV-therapy ineffective in many patients.

The aim of this sub study is to describe the prevalence of HIV-1 subtypes and the patterns of drug resistance mutations among ARV-naïve HIV-1-infected patients from six different countries participating in the GARDEL Study.

MATERIALS AND METHODS

543 naïve patients from 6 countries (Argentina, Chile, Spain, Mexico, Peru and US) were screened between Dec-2010 to May 2012, and 534 HIV-sequences were analyzed following the IAS-USA 2014 Drug Resistance Mutations Panel. Genotypic assays performed at screening visit were: PhenoSense HIV assay (Monogram Biosciences, San Francisco, CA, USA), ViroSeq HIV-1 (ViroSeq HIV-1 Genotyping System v2.0; Celera, Alameda, CA), TRUGENE® HIV-1 Genotyping Assay (Siemens Healthcare Diagnostics), according to availability at each site.

RESULTS

Of the 534 patients screened, 74% were Hispanic/Latino. Median time of infection at SCR was: 10.5 months. CDC stage A: 82%. Of 450 viral subtypes available, the most frequent was subtype B in all three regions (Fig 1). A total of 113 samples (21.2%) had major resistant mutations; 22 samples (4.1%) had major protease mutations (M46I was the most common mutation: 1.5%), 85 samples (15.9%) had NNRTIs mutations (K103N/S was the most common mutation: 4.9%), and 17 samples had mutations to NRTIs (3.2%) M41L (1.3%) was the most common mutation to PIs, only 2 patients had more than one mayor mutation (2/22) (Fig 2). The more frequent minor mutations were: M36I/L/V (21/534), L63P (120/534), L10I/F/V/R (115/534) and K20R/M/I:59/534. The global resistance analysis by regions showed 21% for LA, 22.8% for US/Mexico and 14.7% for Spain, being NNRTI resistance by regions 16.4%; 15.4% and 11.8% respectively. PI resistance was 3.1% for LA and Mexico/US and NRTI resistance was 3.1% for LA, 3.4% for US/Mexico and 2.9% for Spain. No Q151M, 69ss or K65R were identified. (Fig 3)

CONCLUSIONS

In our study we found a primary resistance rate of 21.2%, similar in LA and US/Mexico but lower in Spain. Levels of NNRTI resistance are similar in the three analyzed regions, as previously reported in naïve populations, and reinforces the need of performing genotypic testing in ARV naïve patients, especially in LA were the first line therapy is still based on NNRTI drugs.

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The highly effective antiretroviral therapy has changed the natural history of HIV/AIDS, delaying the disease progression and improving the quality of life of the infected individuals. In treated HIV-1 infected population in Cuba, several factors might have contributed to high drug resistance levels such as prescription of suboptimal regimens containing non-boosted PI, prolonged exposure to failing therapies or due to limited access to laboratory monitoring and limited options for antiviral drug substitutions if required. This might also result in the subsequent spread of drug resistant strains. The performed studies in untreated population have shown high levels of HIV resistance to the antiretroviral therapy ranging from 12% to 23%. The aim of this study is to determine the levels of primary HIV drug resistance in newly diagnosed patients Cubans on a representative sample of the country.

**INTRODUCTION**

The prevalence of antiretroviral drug resistance in HIV-1 infected patients was determined using 263 plasma samples from newly diagnosed patients in Cuba. The present study is a cross-sectional study and gives an overview of the current state of HIV drug resistance in Cuba. The study was conducted in the years 2013-2014. The main objective of the study was to assess the prevalence of transmitted drug resistance (TDR) in newly diagnosed patients in Cuba and to identify the factors influencing the detection of high levels of resistance in newly diagnosed patients. The study also highlights the need for resistance testing in patients who are starting the therapy.

**RESULTS**

The most common mutations associated with resistance to NRTI were M184V (24.2%) followed by thymidine analogue mutations (TAMs) such as D41Y (12.1%), M41L (9%), D67N (6%), M41L (6%). For NNRTI, K103N (45.4%), Y181C (30.3%) and G190A (9.1%) were the most prevalent mutations. The most prevalent PI was D30N (6%).

The detection of a mutation transmitted to ARVs was associated with VL over 100,000 copies/mL ($p = 0.025$, OR = 2.464 (1.148 - 5.288)). The most common resistance mutations associated with resistance to NRTI and NNRTI were TAMs such as L215Y (12.1%), K219N/Q (9%), and G190A (9.1%) were the most frequent mutations (Bennett ET al., 2009). The most frequent subtype detected (28%) was CRF20-23-24_BG, followed by CRF19_cpx (20%) and CRF18_cpx (15.4%). Conversely to that reported so far in the Cuban epidemic, where subtype B was the genetic form predominating, the BG recombinants resulted the most frequent subtype detected (28%), followed by subtype B (24%), CRF19_cpx (20%), the unique recombinant forms (URF) (11%) and CRF18_cpx (10%), although other subtypes were also present.

From the 33 patients with TDR, 22 (66.6%) were HSH, 26 (78.8%) were diagnosed with a recent HIV-1 infection, 13 (39.4%) are from the eastern region of Cuba (29.6%), the highest number of analyzed samples was from Havana with 38.6%, followed by the region of the country (14.8%). The median CD4 cell count value was 371 cells/mm$^3$ (270-573). In the 17.4% (33/189) of the studied samples, transmitted resistance mutations were detected. Simple non-nucleoside mutations contributed the highest amount (45.5%), followed by double class resistance against NRTI and NNRTI (27.3%) and single mutants to the PRI (12.1%).

This study confirms the high levels of resistance in untreated population, it demonstrates the commitment of first-line therapies used in the country and could put at risk future therapies to keep or increase these figures. It highlights the need for studies to elucidate the factors that are influencing detected high levels of resistance in newly diagnosed population in order to take action or to correct the behavior or factors involved in the phenomenon. It also shows the need for resistance testing in patients who are starting the therapy.
Genotypic resistance testing is paramount for the monitoring of the emergence of antiretroviral drug resistant virus. The Viroseq HIV-1 genotyping system v2.0 is an IVD assay for sequencing of HIV-1 from plasma but only feasible if the viral load is at least 1000 cp/mL. However, some patients have a persistent low HIV-1 viraemia inferior to 1000 cp/mL, being resistance testing and antiretroviral therapy hampered by this. So, for their clinical management, resistance testing solutions must be made available. With this regard we developed an in house assay, adapting the Viroseq v2.0 with a nested-PCR protocol.

Blood samples from 36 patients on HAART with a viral load between 20 cp/mL and 1000 cp/mL (range 36-934 cp/mL; mean = 357 cp/mL) were collected in K3EDTA and the plasma separated 6 h after sampling and stored at -80°C. HIV-1 was concentrated by centrifugation of 1 mL of plasma at 24,000g for 1 h at 4°C. After removal of the supernatant, 1 mL of plasma was added and the sample thoroughly homogenized. RNA extraction was performed in the QIASymphonySP equipment from QIAGEN (Hilden, Germany) using the QIASymphony Virus/Pathogen Mini Kit and an in house protocol, rendering a final volume of 30 μL. The Viroseq protocol was performed according to the manufacturer instructions, followed by a nested -PCR protocol based on the previously described by N. Mackie et al. The 50 μL PCR mix contained 0.5 μM of each primer, 1x Incomplete NH₄⁺ Reaction Buffer (DFS-Taq DNA Polymerase – Bioron Life Science), 0.2 mM of deoxyribonucleotide, 2.5 Units of DFS-Taq DNA Polymerase and 5 μL from the products of the first PCR. The PCR was performed on a Perkin Elmer PE9700 thermocycler and consisted on an initial denaturation for 5 min at 95°C, followed by 40 cycles of 95°C for 30s; 55°C for 30s, 72ºC for 120s and a extension at 72°C for 7 min. PCR products were sequenced on the 3130x DNA Analyzer (Applied Biosystems) and analyzed in Viroseq v2,8.

Genotypic resistance testing is essential for the monitoring of the emergence of antiretroviral drug resistant virus being necessary the development of assays for patients with low viral loads.
The role of Presepsin (sCD14-ST) as an indirect marker of microbial translocation and immune activation

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1) Dipartimento di Malattie Infettive, Fondazione IRCCS Policlinico San Matteo, Pavia.
2) Unità di Virologia Molecolare, S.C. di Microbiologia e Virologia, Fondazione IRCCS Policlinico San Matteo, Pavia

Background

- Presepsin is a newly discovered soluble fragment of CD14 studied as a sepsis biomarker.
- The mechanism of its secretion is involved in the TLR4 activation cascade and it is related to mCD14 and sCD14, which are monocyte activation markers, indirectly representing the presence of bacterial translocation. Therefore Presepsin could be employed as an immune activation marker, and it could allow for the estimation of bacterial translocation rates (1).
- The aim of this study was to assess the correlations between Presepsin serum concentration and bacterial translocation, immune activation and fibrosis markers in subjects with HIV and HCV mono-infections and in HIV/HCV co-infection, compared to healthy controls.

Methods

- This is a cross-sectional study included 80 subjects followed up at the Department of Infectious Diseases of Policlinico San Matteo, Pavia University.
- The study population included patients with HIV mono-infection (n = 20), HCV mono-infection (n = 20), HIV/HCV co-infection (n = 20), and healthy controls (n = 20). Peripheral blood was analyzed to determine the levels of Presepsin, Forkhead box 3 (Foxp3+) T cells, TGF-β1, CD14 (soluble and surface isoforms), IL-17 and bacterial translocation products.
- These measurements were correlated to the severity of liver fibrosis, measured with the FIB-4 score and transient elastography.

Results

- Presepsin concentration was significantly higher in the HIV patients (HIV monoinfected and HCV / HIV co-infected). The same group showed increased levels of sCD14 and mCD14, expression of immune activation.
- Statistical analysis show a significant correlation between presepsin and both forms of CD14 only in HIV / HCV group, where the percentage of bacterial translocation and chronic inflammation is high, as shown by the significant increase in bacterial DNA levels, sCD14, mCD14 and IL-17. Presepsin is associated to FIB4 values in the HCV group.

Conclusion

Presepsin is a biomarker of chronic immune activation, as demonstrated by its correlations with sCD14, mCD14 and CD4+CD25+Foxp3+ lymphocytes, particularly in HIV infection. Its concentration is correlated to liver fibrosis markers, such as FIB4, particularly in HCV mono-infected patients.

Considering presepsin and a direct correlation between the levels of fibrosis and an inverse correlation with Treg cells in this group, the low levels of Treg cells may be involved in increasing the state fibrosis in chronic HCV patients.

Reference

CRF19_cpx variant emergence in a cluster in naïve patients of southern Spain. Clinical and phylogenetic characterization

González-Domenech, CM1; Viciana, I1,2; Mayorga, M2; Palacios, R1,2; de la Torre, J1; Jarilla, F1; Castaño, M1; del Arco, A1; Márquez, M1,2; Clavijo, E1; *Santos, J1,2
1Instituto de Investigación Biomédica de Málaga (IBIMA), Spain; 2Hospital Virgen de la Victoria, UGC Infectious Diseases and Microbiology, Malaga, Spain; 3Hospital Carlos Haya, Infectious Diseases, Malaga, Spain; 4Hospital Costa del Sol, Infectious Diseases, Marbella, Spain; 5Hospital de Antequera, Internal Medicine, Malaga, Spain.

Background

HIV CRF19_cpx has been described as a highly pathogenic recombinant from Cuba [1]. Furthermore, these infections are typically associated to higher viral load (VL) at diagnosis and rapid progression to AIDS [2]. Here, we describe the emergence of this CRF19_cpx variant in southern Spain, clustering in men having sex with men (MSM).

Material and Methods

- The study was undertaken at the Virgen de la Victoria Hospital, a reference center for the analysis of HIV-1 genotypic drug resistance in Malaga (Spain).
- Genotypic test was performed in 2298 naïve patients from four hospitals in 2011-2016.
- The subtype for each FASTA sequence provided was assigned through REGA v3.0. Sequences consigned as a CRF19_cpx variant (Fig. 1) were confirmed by phylogenetic analysis with other 195 reference sequences retrieved from LANL.
- Protease and reverse transcriptase (RT) genes were aligned by ClustalX and the phylogenetic reconstruction inferred by maximum likelihood method (RAxML).
- The reliability of the clades was supported on bootstrapping, with 1,000 replications.
- For analysis of RT and protease resistance mutations Standford algorithm v7.1.1 was used.
- Additionally, we collected epidemiological, clinical and immunovirological data.

Results

49 cases
Prevalence: 2.1%

Table 1. Characteristics of the 49 cases

<table>
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<td>HIV transmission</td>
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</tr>
<tr>
<td>No studies/primary school</td>
<td>6 (12.2)</td>
</tr>
<tr>
<td>Undergraduate</td>
<td>21 (42.9)</td>
</tr>
<tr>
<td>University</td>
<td>13 (26.5)</td>
</tr>
<tr>
<td>Origin</td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>46 (94.0)</td>
</tr>
<tr>
<td>Argentina</td>
<td>2 (4.0)</td>
</tr>
<tr>
<td>France</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>Seroconversion time (months)*</td>
<td>24.8 (10.8-21.0)</td>
</tr>
<tr>
<td>Lymphocyte CD4 nadir (cel/μl)</td>
<td>361 (254-416)</td>
</tr>
<tr>
<td>Baseline VL (log copies/ML)**</td>
<td>4.9 (4.5-5.4)</td>
</tr>
<tr>
<td>Baseline lymphocyte CD4 (cel/μl)**</td>
<td>388 (259-470)</td>
</tr>
<tr>
<td>AIDS cases</td>
<td>3 (6.1)</td>
</tr>
<tr>
<td>Death</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>Antiretroviral therapy***</td>
<td>46 (93.9)</td>
</tr>
</tbody>
</table>

*21 cases
**Baseline VL was lower in patients with G190A mutation (4.6 vs 5.1; p<0.02).
***All the patients treated with first-line ART regimens responded.

The quantitative variables are expressed as median (IQR) and the qualitative variables as n (%). MSM: Men sex with men; HTX: heterosexuality.

Conclusions

1. CRF19_cpx variant has emerged affecting MSM naïve patients from southern Spain.
2. All cases but one are related to a local cluster.
3. Half of patients showed the G190A resistance mutation.
4. Unlike previous studies, the variant from Malaga seems less pathogenic, with few cases of AIDS and excellent response to ART.

References

One-Step Real-Time PCR for HIV-2 group A and B RNA plasma viral load in LightCycler 2.0

Pedro Bastos¹, Fátima Monteiro¹, Gilberto Tavares¹, Marina Ferreira¹, Ana Amorim¹, Carolina Rocha¹, Dina Horta±, Claudia Vaz¹, Rosário Serrão², António Sarmento², Fernando Araújo³, M. Carmo Koch¹
¹ Molecular Biology Center, Blood Bank and Transfusion Department, Hospital S. João, Porto, Portugal.
² Infectious Diseases Department, Hospital S. João, Porto, Portugal.

Background
Although with a lower prevalence than HIV-1, HIV-2 is responsible for localized epidemics, being Portugal the non African country with the greatest expression of the infection. Clinical management of the infection is hampered by the lack of validated commercial RNA viral load assays, thus their in house development using the available equipment is mandatory.

Material and Methods

Samples
HIV-2 was confirmed by Innolia™ (Innogenetics, Gent, Belgium). Blood samples were collected in K₃EDTA and the plasma separated 6 h after sampling and stored at -80°C. The BIOQ HIV-2 RNA group A quantification panel (Biocentric, Bandol, France) was used as an external standard.

HIV-2 RNA isolation
RNA extraction was performed from 1000 μL of plasma in the QIASymphonySP (QIAGEN, Hilden, Germany) using the QIASymphony Virus/Pathogen Mini Kit and a generic protocol, rendering a final volume of 60 μL. RNA from the samples and standards was isolated under the same conditions.

HIV-2 RNA quantification (RT-qPCR)
The protocol was based on the previously described by Avettand-Fenoel et al.⁴ Primers and probes are described on table 1. The one step RT-qPCR was performed on the LightCycler 2.0 (Roche Diagnostics, Mannheim, Germany) with the Lightcycler RNA Virus Master kit from Roche (Roche Life Sciences, Mannheim, Germany) was used. The 20 μL reaction mixture contained 0.5 μM of each primer, 0.25 μM of each probe, 0.4 μl of Enzyme Blend and 7.5 μL of the isolated RNA. RT-qPCR cycling conditions consisted on 10 min at 60°C and 60s at 95°C, followed by 50 cycles of 95°C for 5s; 60°C for 50s and 72°C for 10 min.

Results
The standard curve generated by the LightCycler software (version 4.05) presented an efficiency of 2.079 (103%), an error of 0.0657 and a r² of 1.0 (Fig. 1). Besides the detection of B subtypes, this RT-qPCR provides a linear range between 5.03 x 10⁶ cp/mL and 5.03 x 10² cp/mL, adequate to the low HIV-2 viral loads in plasma.

To evaluate repeatability and reproducibility, clinical samples tested with the previous method and serial dilutions (10⁵-10² cp/mL) of the NIBSC HIV-2 NIH-Z strain (Advanced Biotechnology Incorporated, Columbia, Maryland) were tested in replicates (Fig. 2) in the same and in independent runs with different operators, with a CV lower than 0.28 and SD lower than 0.8 (data not shown).

Conclusion
This assay allows us to evaluate HIV-2 A and B subtypes viral load in plasma with satisfactory sensibility and reproducibility, supporting the clinical management of the infection.

Table 1. Primers and Probes sequences.

<table>
<thead>
<tr>
<th>Primers and Probes</th>
<th>LTR region</th>
<th>gag region</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTR F</td>
<td>5'-TCTTTAAGCAAGCAAGCGTG-3</td>
<td>5'-GCGCGAGAACTCCGTCTTG-3</td>
</tr>
<tr>
<td>LTR R</td>
<td>5'-AGCAGTAGGAAGCTGGGTGTT-3</td>
<td>5'-TTCGCTGCCCACACAATATGTT-3</td>
</tr>
<tr>
<td>LTR P</td>
<td>5'FAM-CTTGGCCGGYRCTGGGCAGABHQ1-3</td>
<td>5'FAM-TAGGTTACGGCCCGGCGGAAAGA-BHQ1-3</td>
</tr>
<tr>
<td>gag F</td>
<td>5'-CCGAGCCGGCTAACCTTG-3</td>
<td>5'-CCGAGCCGGCTAACCTTG-3</td>
</tr>
<tr>
<td>gag R</td>
<td>5'-CCGAGCCGGCTAACCTTG-3</td>
<td>5'-CCGAGCCGGCTAACCTTG-3</td>
</tr>
</tbody>
</table>

References
The association between high pre-HAART CD8 counts and poorer immunological outcome following antiretroviral therapy

Bonnie Wong¹, Ngai Sze Wong², Shui Shan Lee²

¹Integrated Treatment Centre, Centre for Health Protection, Department of Health, Hong Kong
²Stanley Ho Centre for Emerging Infectious Diseases, The Chinese University of Hong Kong

Background

• Nadir CD4 counts, advanced age and hepatitis C co-infections are known predictors of poorer immune recovery following highly active antiretroviral therapy (HAART)
• CD8 counts could be another useful marker for prognostic monitoring as high CD8 was associated with inflammatory non-AIDS-related clinical events
• We aimed to examine the association between pre-HAART CD8 and immunological outcome among patients on HAART for ≥4 years

Methods

• Data source: anonymous clinical data from Integrated Treatment Centre, the largest HIV service in Hong Kong
• Eligibility: adult HIV+ patients, with available negative HIV testing result within 3 years before HIV diagnosis, treatment-naïve, on HAART continuously for ≥4 years
• Optimal immunological outcome: CD4>500/μL & CD4/CD8 ratio>0.8
• Statistical analysis: multivariable logistic regression with baseline CD4 count as confounder

Results

• n=199
• Age: median 36 years old, IQR 31-41 years old
• Male: 180 (90%)
• Chinese: 165 (83%)
• Mode of transmission:
  - Heterosexual: 80 (40%)
  - Men who have sex with men: 116 (58%)
• Interval from diagnosis to latest assessment: median 12.7 years, IQR 9.4-17.4 years
• Regimen:
  - protease inhibitor (PI)-based (70%)
  - non-nucleoside reverse transcriptase inhibitor (NNRTI) based (30%)
• Treatment duration: median 78 months, IQR 57-112 months

Table 1. Comparison between patients with high (>800/μL) and low (≤800/μL) pre-HAART CD8 counts

<table>
<thead>
<tr>
<th></th>
<th>pre-HAART CD8 &gt;800 (n=96)</th>
<th>pre-HAART CD8 ≤800 (n=101)</th>
<th>Odds ratio (OR) adjusted by pre-HAART CD4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>median/ IQR/ %</td>
<td>median/ IQR/ %</td>
<td></td>
</tr>
<tr>
<td>Baseline characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td>86/90%</td>
<td>92/91%</td>
<td>1.6</td>
</tr>
<tr>
<td>Chinese ethnicity</td>
<td>79/84%</td>
<td>85/85%</td>
<td>1.6</td>
</tr>
<tr>
<td>Estimated cumulative viral load *</td>
<td>17.74/10.00-29.61</td>
<td>18.53/10.88-27.73</td>
<td>1.004</td>
</tr>
<tr>
<td>NNRTI-based initial regimen</td>
<td>29/30%</td>
<td>28/28%</td>
<td>1.52</td>
</tr>
<tr>
<td>Outcome at Year 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 count /μL</td>
<td>454/339-553</td>
<td>431/328-561</td>
<td>1.002</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>0.49/0.36-0.61</td>
<td>0.61/0.42-0.82</td>
<td>298.28</td>
</tr>
<tr>
<td>Viral load (log10)</td>
<td>1.88/1.88-2.65</td>
<td>1.88/1.88-2.65</td>
<td>0.55</td>
</tr>
<tr>
<td>Suppressed viral load ≤500 copies/mL</td>
<td>91/96%</td>
<td>99/99%</td>
<td>4.28</td>
</tr>
<tr>
<td>CD4≥500/μL &amp; CD4/CD8 ratio ≥0.8</td>
<td>9/9%</td>
<td>23/23%</td>
<td>6.64</td>
</tr>
<tr>
<td>Treatment (months)</td>
<td>32/25-36</td>
<td>26/20-39</td>
<td>0.96</td>
</tr>
</tbody>
</table>

*Estimated cumulative viral load from seroconversion to pre-HAART expressed as years; log10 viral load copies/mL
*p<0.05

Acknowledgement:
The study was supported by the Health and Medical Research Fund (CU15-A15) of Food and Health Bureau of the Hong Kong Special Administrative Region Government. Technical support was provided by Li Ka Shing Institute of Health Science, The Chinese University of Hong Kong.

Conclusion

• A pre-HAART CD8 count of >800/μL gave a high odds of poorer immune outcome
• Pre-HAART CD8 count is an independent predictor of an outcome measure comprising CD4 count and CD4:CD8 ratio
• While CD4 is a useful prognostic marker, the strength of prediction increased with the addition of baseline CD8 count using a cutoff of 800/μL.